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Editor

HAMILTON B. G. ROBINSON

JUNE, 1948

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Endowment fund of the International Association for Dental Research (as of Mar. 16, 1946): $1,402.98. There have been no expenditures from this fund.

International Association for Dental Research

Twenty-Sixth General Meeting

June 19 and 20, 1948

Rochester, N. Y.

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THE EDITOR'S VIEWPOINT

Blayney and Tucker (p. 279) give the purpose and mechanism of the Evanston Dental Caries Study. This interesting study is being carried out in Evanston and Skokie, Illinois, and being controlled by studies in Oak Park, Illinois, in order to evaluate the use of fluorine in the water supplies as a method of dental caries control. The outline of the study, which is projected over fifteen years of time, is quite complete and should serve as an excellent example to others who may be planning tests of sodium fluoride added to the water supplies of urban communities. The children will be examined at regular intervals, the water supply is carefully controlled, and an estimation has been made of the cost of the use of sodium fluoride. For the first year, the cost of sodium fluoride was 10.7 cents per capita. It is hoped that the data from this study will be available from time to time so that any early effects may be evaluated by other workers.

McClure (p. 287) has reviewed the literature on the fluorine content of dentin and enamel of sound and carious human teeth. The review of the literature indicated, as a general rule, the data are insufficient to define the relationship between fluorine content and mottled enamel of individual teeth. Studies on teeth from thirty-three individuals, studied separately, and two groups of fourteen and forty-four individuals, give interesting results on fluorine content of the enamel and dentin. The differences between the fluoride of enamel and dentin from sound and carious teeth was significant. These data do not substantiate the theory that there is more fluorine in caries-resistant teeth than in caries-susceptible teeth, but there is not enough evidence to say whether or not an optimum quantity of fluorine in the dentin and enamel of the entire dentition is associated with the reduced incidence of dental caries. Further data are needed for demonstration of this possibility and such an investigation is promised by the author.

Hadjimarkos and Storvick (p. 299) report the incidence of dental caries among Freshman students at Oregon State College. Dental examinations were made on 582 students. Their caries histories were compared with those of subjects of the same age examined in other geographic areas. The Oregon State students were studied by visual and explorer examination and posterior bite-wing radiographs. The dental caries experience among the Oregon State students seems high in comparison with that in other geographic areas of the country; the female students had a higher D.M.F. rate and the teeth of the female students had received greater care. This study is an interesting and careful study of limited data and suggests the possibility for careful accumulation of data by clinicians for analysis of the caries stituation. Isolated studies may appear to have little validity but when grouped together begin to create a pattern which may suggest some of the factors associated with caries resistance.

Dreizen and Spies (p. 305) have made further studies of the association between products of protein putrefaction and dental caries. They found that dilute solutions of skatole, phenol, cresol, and a salt of putrescine partially or completely inhibited acid production in vitro in saliva samples. The action of these compounds appeared to be additive, and they were most effective as inhibitors of acid production in saliva when used in combinations. Pure cultures of *Lactobacillus acidophilus* were inhibited by the use of these compounds. The study supports the hypothesis that the products of protein putrefaction may prevent the growth of acidogenic organisms associated with dental caries. The authors offer this as a partial explanation of the lower incidence of dental caries associated with pellagrins and individuals with chronic nutritional deficiency diseases. The relationship of putrefaction and dental caries activity is an interesting phase of the caries problem and probably justifies investigations being carried out from different viewpoints.

Kitchin and Permar (p. 316) report the results of a three-year study of the effectiveness of carbohydrate restriction in reducing *Lactobacillus acidophilus* counts. Careful analysis of specimens from 559 individuals

whose saliva specimens were subjected to *L. acidophilus* counts showed 137 individuals who were caries inactive and 422 who were caries active by this test. The studies indicate that the *L. acidophilus* count in the saliva may be lowered by restricting the sugar intake and the results compare favorably with those presented by the Michigan group. There is need for recording the results of treatments such as this so that their effectiveness may be evaluated against other technics. The results in this study should be compared with those reported by Becks (p. 405 as part of the Michigan Proceedings Conference in this issue of the JOURNAL.

Brudevold (p. 320) has applied the Hoffman's modification of the Fisk and Subbarow method for phosphate analysis to a study of the phosphate solubility of human enamel surfaces. Variations in phosphate solubility were found from area to area of the same tooth and between various areas on different teeth. Deciduous and unerupted permanent teeth were more soluble than intact enamel surfaces. This method might well be applied to studies of the effect of fluorides and other compounds on the solubility of teeth rather than using studies based on powdered enamel which are not as comparable to the in vivo condition.

Mitchell (p. 330) describes the normal and diseased periodontium of the hamster. This useful laboratory animal which has become so popular in dental caries studies appears to offer a useful laboratory tool for study of periodontal disease. A form of periodontal disease appeared among stock animals and was produced in normal young animals by interfering with normal conditions. The hamster is recommended by Mitchell as a tool for studying periodontal lesions in the laboratory.

Annis (p. 336) presents a theory on the conduction of pain from the surface of the tooth to the central nervous system. His theory, which he terms the electrochemical theory, proposes that pain is conducted as electrochemical impulses from conditioned enamel and dentin to the pulp. The author suggests that his theory explains many clinical observations that have not been explained by earlier theories. A series of brief experiments are outlined that seem to confirm some of the author's ideas. The work was carried on by an investigator who is not associated with any university center or any other institution where laboratory facilities are readily available and demonstrates the possibility of investigations being carried out on an individual basis if one has the will to carry on such work.

The proceedings of the Michigan Dental Caries Conference are concluded in this issue. We believe that this brings together a mass of data and opinion which should be of value to all students of dental caries and which should clarify many doubts which have been present in the minds of dentists and teachers concerning the thoughts of the investigators in this particular field. We have departed from custom in reprinting the reports of the Evaluating Committees which have previously been published in the *Journal of the American Dental Association*. The *Journal of the American Dental Association* kindly granted permission to do this and it was carried out because it was felt that this was necessary to complete the studies in one journal. The entire proceedings were printed without reduction in the normal pages of each issue and this volume will be longer by the number of pages included in the proceedings. It is still our hope that our publisher and the University of Michigan may get together to make this material available in a single bound volume so that students and others may receive the advantages of this very worthwhile discussion.

H. B. G. R.

THE EVANSTON DENTAL CARIES STUDY

II. Purpose and Mechanism of the Study

J. R. BLAYNEY, D.D.S., AND WINSTON H. TUCKER, M.D.

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DURING the past few years, public health workers have become interested in the problem of oral disease. It is now generally recognized that tooth decay cannot be controlled by individual treatment. Ways and means must be developed for the control of tooth decay so that entire communities will benefit.

Recognizing this need for mass control of tooth decay, the Commissioner of Health of Evanston, Illinois, called a meeting of the dental and medical societies in his community. An advisory committee was appointed to confer with him about the problem of community oral health. Because of the interest shown by the advisory committee in the possible relationship of fluorine to the prevalence of dental caries, Dr. H. T. Dean, Director, Dental Division, United States Public Health Service, was invited to address a joint meeting of the local physicians and dentists on Dec. 11, 1944.

After further deliberation of the project, both professional groups recommended to the Commissioner of Health that a carefully controlled study be developed to determine whether or not the addition of fluorine in minute quantities to the communal water supply would reduce the incidence of dental caries in Evanston and Skokie children. The advisory committee and Commissioner of Health then discussed the problem with members of the Walter G. Zoller Memorial Dental Clinic. Because the success of such a program depends upon the enthusiastic support of many different groups, a meeting was held with representatives of each. It was carefully explained to these Evanston citizens that nothing could be promised regarding the ultimate value in the control of tooth decay; that if such a program was to be undertaken it must be in the nature of an exhaustive study; and that it would be several years before data would be available which would even indicate the trend which we might expect.

As a result of this meeting, the Evanston City Council on Nov. 5, 1945, passed a resolution authorizing the study and the purchase of the equipment for the addition of sodium fluoride to the city water. Because the Village of Skokie purchases its water supply from Evanston, a similar resolution was passed by the officials of that community on May 7, 1946. The Director of the Illinois State Department of Health gave his approval; the public and parochial school officials pledged their wholehearted support; and the medical and dental professions endorsed the project. With this magnificant cooperation, the following program was finally evolved.

Received for publication March 4, 1948.

PROGRAM

The method by which sodium fluoride acts to reduce the incidence of dental caries is not definitely known. Therefore, it was considered advisable to plan the study in a manner so as to provide as much information as possible. It was our opinion that the school children in the 6-, 7-, 8-, and 12-, 13-, 14-year age groups will provide the best opportunity to assess the caries experience in the deciduous dentition and to study the initial caries attack rates of the permanent teeth. Furthermore, choosing these age groups will provide the opportunity of appraising the rate at which caries progresses in the various age levels during different time intervals following the introduction of fluoride into the water supply. In addition, we shall have the opportunity to compare our findings with those of several other studies in which similar age groups were employed.

It also seemed advisable to plan to continue the study for a fifteen-year period. When at least, one group reaches the age of fourteen (maximum in this study) the children will have been exposed to fluorine throughout their entire life, that is prenatal and postnatal. The schema, Fig. 1, indicates the groups to be examined each year; those age groups that from time to time will be re-examined; and the many opportunities for comparing the caries incidence in each age group after different time intervals of exposure to fluorine.

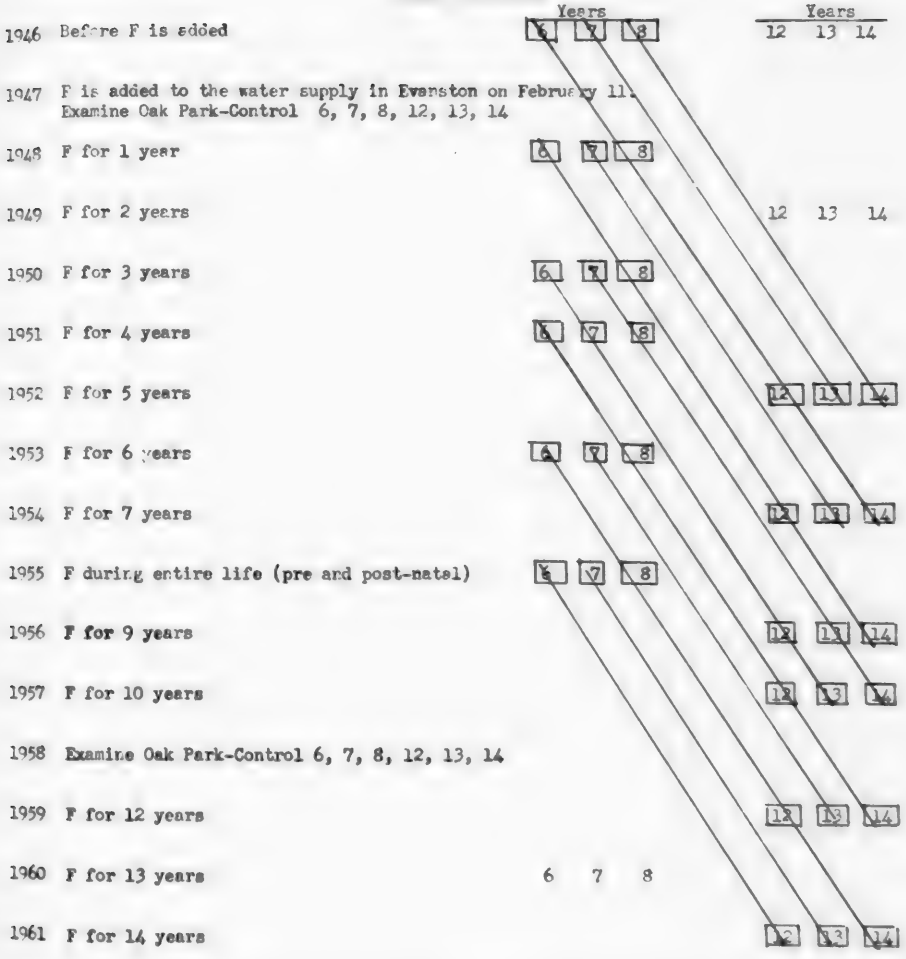
A study of this nature must have an adequate control. It seemed logical to think of Oak Park, Illinois, as the ideal control community because of its close similarity to the study area. After careful consideration, the dental and medical professions, the Department of Health, and the officials of the public elementary schools and the high school of Oak Park graciously offered to serve as the control community.

It is necessary to support the clinical findings with information which cannot be obtained from the child. Therefore, a questionnaire was prepared which is sent to the parents and returned to the home-room teacher (Fig. 2). The data are transferred to the permanent history card prior to the clinical examinations. All questions are considered essential in the evaluation of the oral condition of each child. However, some of the more important questions are: (1) other places of residence with the dates of each; (2) vacations or obsences from Evanston, Skokie, or Oak Park of more than thirty days at a time, with dates and places of such residence; (3) the place of residence of mother during gestation; (4) special diets of mother during pregnancy; (5) the dietary habits of the child, and (6) kinds and periods of illnesses of the child.

SCHOOL CLINICS

Dental clinics are established in certain public and parochial schools which are selected because of their physical facilities and because of their convenient location. Children are transported from near-by schools to the dental clinic in order to reduce the frequency of moving the clinic. The dental equipment consists of two dental chairs used for the oral examinations, two dental chairs for the x-ray survey, two mobile type x-ray machines, two Pelton operating lights,

SCHEMA FOR STUDY


Years
6 7 8
Years
12 13 14
1946 Before F is added
1947 F is added to the water supply in Evanston on February 11.
Examine Oak Park-Control 6, 7, 8, 12, 13, 14
1948 F for 1 year
1949 F for 2 years
1950 F for 3 years
1951 F for 4 years
1952 F for 5 years
1953 F for 6 years
1954 F for 7 years
1955 F during entire life (pre and post-natal)
1956 F for 9 years
1957 F for 10 years
1958 Examine Oak Park-Control 6, 7, 8, 12, 13, 14
1959 F for 12 years
1960 F for 13 years
1961 F for 14 years

Connecting lines indicate children who will be re-examined and age at time of examination.

Fig. 1.

DENTAL CARIES STUDY—EVANSTON, ILLINOIS

Name _____ (Last / First)

Sex ..... Color ..... Color hair ..... Color eyes .....

Home address ..... Telephone No. ..... School .....

Date of birth ..... (Mo. Day Year) Birthplace ..... (Town and state) Lived in Evanston since ..... (Date)

List dates and other places of residence ..... (Designate town and state. Indicate Town (T) or Farm (F))

Check type of water supply in above places: Well water ..... Cistern water ..... City water ..... Spring water .....

Vacations or absences from Evanston ..... (More than 30 days at one time. Places of residence during that time with date)

Do you now use city water supply? ..... Bottled water for drinking? {Yes ..... No .....} for cooking? {Yes ..... No .....} Brand ..... (Bottled water)

Did mother live in Evanston during pregnancy? Yes ..... No ..... If not, where? ..... (During what mos. of pregnancy)

Was mother seriously ill during pregnancy? Yes ..... No ..... If so, name illness .....

Did mother take: Calcium compound? Yes ..... No ..... ; special cereal or bone meal? Yes ..... No ..... If so, please state kind .....

Are there any members of family with impaired hearing? Yes ..... No ..... If so, who .....

DIETARY RECORD OF CHILD:

Breast-fed? ..... How long? ..... Cod-liver oil or vitamin D concentrate? Yes ..... No .....

Age started ..... Orange juice or Vitamin C? Yes ..... No ..... Age started .....

Cereal ..... Kinds ..... (Since birth) How long? .....

Does child have a good appetite? Yes ..... No ..... List below foods child does not like and seldom eats .....

Do you use a pressure cooker for cooking vegetables? Yes ..... No .....

Does child eat between meals? Daily ..... Frequently ..... (3-4 times per week) Occasionally ..... (1-2 times per week) Rarely ..... (1-2 times per month)

What? .....

Does child use the following?	*Daily*	*Frequently* (3-4 times per week)	*Occasionally* (1-2 times per week)	*Rarely* (1-2 times per month)
Cod-liver oil or vitamin D				
One quart milk				
Citrus fruits and/or tomatoes				
Chewing gum				
Candy, jam, or jelly				
Soft drinks				
Pastries {Cookies, cake, pie, sweet rolls}				

List childhood diseases or accidents: Age

.....

.....

.....

Has child taken calcium in some form under doctor's orders?
Yes ..... No ..... Date .....

Tonsils removed at .....

Child's physician ..... (Name) (Address) .....

Child's dentist ..... (Name) (Address) .....

Date examined _____ Unit No. _____ Serial No. _____

Fig. 2.

List names of other children in family:

Name	Date of birth	Sex

Name of mother..... Birthplace..... (Town and state)

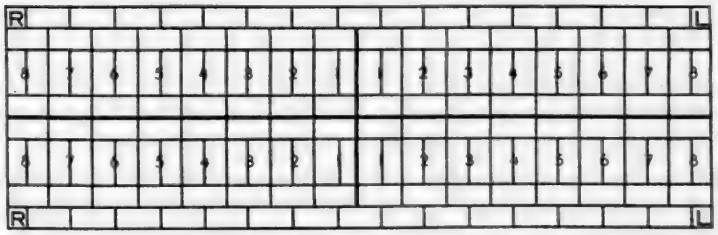
Name of father..... Birthplace..... (Town and state)

Does child do well in school? Yes..... No.....

Does child get along well with playmates? Yes..... No.....

Is child emotionally stable? Yes..... No.....

Remarks:



PROXIMALS
Dec.
Cl. .....
X .....
Perm.
Cl. .....
X .....

20 .....
24 .....
30 .....

Number of carious teeth ..... Filled teeth ..... Carious surfaces ..... Filled surfaces .....

Number new carious teeth ..... Number new carious surfaces in previously decayed teeth .....

Caries index: Dec. D.M.F.T. ..... S ..... Perm. D.M.F.T. ..... S .....

No. extracted teeth ..... No. teeth needing extraction .....

Congenitally missing teeth: Specify ..... Immunity: Questionable ..... Deciduous ..... Permanent .....

Classification of fluorosis: Normal ..... Questionable ..... Very mild ..... Mild ..... Moderate ..... Severe .....

Oral hygiene: Good ..... Fair ..... Poor ..... Calculus: Salivary ..... Serumal .....

Saliva: Normal ..... Abnormal ..... Malformed Teeth .....
(Specify thin, thick, ropy, scanty, profuse, etc.)

Gingival condition: Normal ..... Hyperemic ..... Inflamed ..... Hypertrophic ..... Atrophic ..... Ulcerative .....

Occlusion: Normal ..... Malocclusion ..... (Class I II III) Orthodontic treatment { Past ..... Present .....

Enamel dystrophies:

White opacities ..... Enamel pits and/or defects ..... Mulberry molars .....

Brown opacities ..... Hutchinson's teeth ..... Other anomalies .....

Fractured or chipped incisal edges: Specify: .....

Salivary counts: ..... Physical defects: .....

Deciduous teeth collected for fluorine determination ..... Fluorine content of teeth .....

Blood: Hemoglobin ..... Coagulation ..... Urine: Fluorine content .....

Examiner ..... D.D.S. Transfer to Code Sheet—Incomplete ..... Complete .....

Fig. 3.

an electric sterilizer, an instrument cabinet, tables, desks, and so forth. A complete selection of mouth mirrors, explorer points of several types to permit the examination of all surfaces of the tooth crowns, dental floss, air syringes, cotton rolls, and pellets is provided.

The staff is on a full-time basis and consists of three dentists, two x-ray technicians, a graduate nurse with special training in public health, a chemist, and a secretary. Only two of the dentists make clinical examinations at one time while the third dentist remains at the central office (1) to keep the records up to date, (2) to conduct the laboratory work connected with the bacteriological study of the salivary specimens, and (3) to read the literature pertinent to the study.

All appointments for visits to the clinic are arranged by the clinic nurse cooperating with the school personnel, usually the school nurse. Every effort is made to schedule a child's visit to the dental clinic in accord with the individual school program. Four children are scheduled for the clinic at nine o'clock in the morning and every forty minutes thereafter until noon. A similar forty-minute schedule is maintained in the afternoon until the close of the last school period. This schedule makes it possible to conduct simultaneously two clinical and two x-ray examinations and allows approximately twenty minutes for each. When the patient is properly seated in the dental chair with the mouth well-illuminated and with a wide range of suitable instruments, it is possible to make a detailed examination of the teeth and the investing tissues in the twenty-minute period. The roentgenological examination consists of three apical-view films of the maxillary anterior teeth, three apical-view films of the mandibular anterior teeth, and one bite-wing film each of the right and the left side of the 6-, 7-, and 8-year-old children, and two bite-wing films each of the right and left side of the 12-, 13-, and 14-year-old children. The routine of using eight to ten films per mouth provides ample opportunity to examine critically each proximal surface for early carious lesions unless the teeth are malposed. All films are processed at a standard temperature of 68° F. for a five-minute period which, in our experience, produces a contrast best suited for the recognition of early caries of the enamel. The exposures are made with the machines adjusted so as to deliver approximately fourteen milliamperes.

At the time of the clinic visit, the dentist records on the history sheet the location of all carious lesions found by a careful clinical examination as well as tooth surfaces which previously have been filled, extracted teeth, teeth that need extraction, and many other conditions, as shown in Fig. 3. During the first part of each week stimulated salivary specimens are collected from a random sample of children for the bacteriological study. The specimen is collected before either the clinical or the roentgenological examination has been made. These samples from 10 per cent of those scheduled for examination during the current week are taken during the latter periods of the morning and afternoon so as to insure a uniform lapse of time after eating. The majority of children will provide a 5 to 8 c.c. specimen within five minutes; however, occasionally a longer time is required to collect a sample of suitable volume. Whenever this

occurs, a record is kept of the time required to collect 5 c.c. of saliva. The bottle is placed in an iced Thermos jug in order to control the bacterial growth. All specimens are cultured the same day they are gathered by 0.1 ml. of a 1:10 and a 1:100 dilution of the saliva being spread on plates of modified tomato juice agar adjusted to a pH of 5.2. Two plates are prepared of each dilution. The average count of the two 1:10 dilution plates are considered as the lactobacillus count for that specimen.

Following the last school period, the dental team returns to the central office in the Health Department building in Evanston to complete the day's work. The x-ray technicians process the films taken during the day and the dentists inspect the x-ray films of the children they examined on the previous day to discern very early carious lesions on the proximal surfaces which were not detected by the clinical procedures. All carious lesions visualized in the film and not recorded during the clinical study are noted on the history chart as carious lesions observed by x-ray only. We believe that it is very important to locate and record every site of caries activity at each examination if the study is to be of value.

The clinical study in Evanston and Skokie prior to the addition of fluoride to the water extended from Jan. 28, 1946, to Feb. 10, 1947. The study in Oak Park was instituted on Feb. 26, 1947, and was concluded on Nov. 10, 1947. This base line survey is to determine the prevalence of dental decay in the various age groups prior to the addition of sodium fluoride to the water supply in Evanston and Skokie and will serve as the basis for the comparison with subsequent findings. The Oak Park survey is for the purpose of determining the prevalence of dental decay in the control community at the time the study was instituted and to serve as the basis for comparison with the findings of future surveys both in the control and the study area while the program is in progress. These base line observations were made on 4,375 North Shore children and 2,493 Oak Park children. This required taking and studying 61,378 x-ray films. For the purpose of determining the lactobacillus content of the saliva, we collected and studied 672 specimens. As rapidly as all of the data for each child have been gathered and recorded on the permanent history card the findings are transferred to a code sheet. These code sheets are forwarded to the Bureau of Vital Statistics, State Department of Health, for the transfer to punch cards which will be used to sort and tabulate the data.

A chemical laboratory adequately equipped for accurate fluorine determination has been established in the filtration plant of Evanston. In this laboratory samples of water, which has been gathered daily from several and varied points in the distribution system, are analyzed for their fluorine content. The reports indicate that the fluorine content of the water does not vary more than 0.05+ of one part per million. Besides determining the fluorine content of these water samples, many different types of food purchased in the Evanston markets are examined for their fluorine content. This is for the purpose of providing some information regarding the probable amount of fluorine taken

in the complete diet. Many tooth and bone specimens from persons living within the study area are also examined for their fluorine content. One report giving the findings of these chemical studies has been published.*

The first examination of the 6-, 7-, 8- and 12-, 13-, 14-year-old school children of Evanston and Skokie was completed on Feb. 10, 1947. The addition of sodium fluoride to the water supply was begun on Feb 11, 1947. Through Feb. 10, 1948, a total of 81,375 pounds of sodium fluoride had been placed in the city water. This was accomplished at an estimated cost of $0.1073 per capita.

Arrangements have been completed to begin the second examination of the 6-, 7-, 8-year-old school children of Evanston and Skokie on Feb. 16, 1948. After the study of this group has been completed, we can compare the data with similar groups in the base line study to ascertain if there is any change in the prevalence of tooth decay. It is not expected that significant changes will be observed; furthermore, it probably will be some time before caries will be reduced materially.

The data will be reviewed at the close of each year's program. The findings will be made available to research workers through scientific journals and to the lay public by news releases in the daily press.

*Martin, D. J.: J. D. Res. 27: 27, 1948.

FLUORINE IN DENTIN AND ENAMEL OF SOUND AND CARIOUS HUMAN TEETH

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IN A review of the literature in 1933, enamel, dentin, and whole teeth were reported to contain from 0.0 per cent to 1.4 per cent fluorine.[1] The published results up to that time indicated the presence of 0.10 to 0.20 per cent fluorine in enamel. Following 1933, the accuracy of the fluorine analytical procedure was improved but the quantity of data relative to dental tissues has remained very limited. Additional data on fluorine in human dentin and enamel are desirable, particularly since fluorine has gained much prominence in the prevention of dental caries.[2] The published results on the fluorine content of teeth beginning with the year 1933 are summarized in Table I, *A* and *B*).

While the data given in Table I, *A* are insufficient to define the relation between fluorine content and mottled enamel diagnosis of individual teeth, as a general rule, exposure to fluoride waters, particularly during the formative period, results in more than normal percentages of fluorine being present in both dentin and enamel (See Ockerse,[6] Table I, A and B).

Fluorine determinations, however, are not consistent with respect to fluorine in sound versus carious teeth. The most extensive evidence on this question has been supplied by Armstrong and Brekhus[7] whose data are statistically significant and support the view that enamel of sound teeth may contain almost twice as much fluorine as enamel of carious teeth. These investigators found no differences in dentin of sound and carious teeth. Ockerse[6] reported identical mean values for fluorine in sound and carious enamel and no significant differences in dentin. Restarski,[8] in heretofore unpublished analyses, obtained fluorine values which appear to be somewhat high for both sound and carious dentin and enamel. His data seem to imply a somewhat elevated fluorine exposure by the group of Samoan natives whose teeth he analyzed, and, as he reported,[14] the Samoans have an unusually low caries index.

The mean fluorine content of enamel of nine sound teeth, according to Restarski's data, was 0.0150± .0012 per cent, whereas fluorine in the enamel of fourteen carious teeth had a mean value of 0.0110± .0010 per cent. The difference between enamel of sound and carious teeth, with its probable error, is 0.0040± .0015 per cent fluorine, which is too small to be statistically significant. Aside from the data presented by Armstrong and Brekhus,[7] therefore, there has been no evidence that enamel of carious and noncarious teeth differs in fluorine content. Furthermore, the analytical data thus far give no indication of how fluorine may vary from one type of tooth to another, or in the teeth of one individual as compared with those of another.

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TABLE IA

FLUORINE IN SOUND AND CARIOUS TEETH NOT EXPOSED TO FLUORIDE DOMESTIC WATERS
(DATA PUBLISHED AFTER 1933)

OBSERVER		DENTIN (% F)	ENAMEL (% F)	DESCRIPTION AND LOCATION
Boissevain and Drea[3]		0.0600	0.0250	Sound; New York City
Bowes and Murray[4]	Range	--	0.0-0.0200	Sound; London, Eng.
Bowes and Murray[5]	--	0.0250	0.0250	"Normal"
Ockerse[6]	Mean	0.0268	0.0153	Sound; non-F area; Knysna, S. Africa
	Range	(0.0200-0.0320)	(0.0130-0.0180)	
	Mean	0.0252	0.0153	Carious; non-F area; Knysna, S. Africa
	Range	(0.0140-0.0520)	(0.0070-0.0310)	
Armstrong and Brekhus[7]	Mean	--	0.0160	Sound; 36-year-old man
	Mean	--	0.0070	Carious
	Mean	--	0.0109	Sound; 28-year-old woman
	Mean	--	0.0063	Carious
	Mean	--	0.0112	Sound; sex and age unknown
	Mean	--	0.0067	Carious
	Mean	--	0.0114	Sound; mean of several individuals
	Mean	--	0.0068	Carious
	Mean	0.0169	0.0111	Sound
	Range	--	(0.0073-0.0167)	
	Mean	0.0158	0.0069	Carious
	Range	--	(0.0049-0.0087)	
Restarski[8]	Mean	0.0646	0.0150	Sound; teeth from Samoan natives
	Range	(0.0418-0.1071)	(0.0100-0.0243)	
	Mean	0.0423	0.0110	Carious
	Range	(0.0100-0.0903)	(0.0060-0.0246)	
Roholm[9]	Mean	0.0310*	0.0044*	Normal incisors
	Mean	0.0300	0.0057	Normal molars

*Roholm's figures refer to fluorine in ash.

TABLE IB

FLUORINE IN TEETH EXPOSED TO FLUORIDE
(DATA PUBLISHED AFTER 1933)

OBSERVER		DENTIN	ENAMEL	DESCRIPTION AND LOCATION
Boissevain and Drea[3]	Mean	0.1120	0.0650	From fluoride area*
Bowes and Murray[5]	Mean	0.0730	0.0320	"Mottled teeth"; England
Armstrong and Brekhus[10]	Mean	--	0.0357	Mottled; severe
	Mean	--	0.0333	Mottled; severe
	Mean	--	0.0361	Mottled; moderate
	Mean	--	0.0343	Mottled; questionable
Sognnaes and Armstrong[11]	Mean	0.0196	0.0140	Deciduous; sound
	Mean	0.0270	0.0140	Permanent; carious or malposition From Tristan de Cunha†
Ockerse[6]	Mean	0.0755	0.0445	From Calvinia, S. Africa, a fluoride area‡
	Range	0.0130-0.1700	(0.0150-0.0750)	
Hamersma[12]	Mean	0.0456§	0.0177§	Teeth from 19-year-old boy living in Pretoria, S. Africa, a fluoride area
	Range	(0.0317-0.0680)	(0.0074-0.0285)	
Roholm[9]	Mean	0.0830§	0.0290§	Teeth from worker in cryolite (Na_3AlF_6) factory

*Colorado Springs drinking water which contains 2.5 to 2.6 ppm fluorine.

†A mild fluoride exposure from food is suggested in connection with this study.

‡Ockerse reported 1.5 ppm fluorine in drinking water from this area.[6] He reported also as much as 13.9 ppm fluorine in water from bore holes, used for drinking in some areas in South Africa.[13]

§Data refer to fluorine in ash.

PROCEDURE: TEETH AND FLUORINE DETERMINATION

For obvious reasons the usual diagnosis of a tooth as carious upon the finding of cavities or fillings is not a criterion of active caries. An unfilled cavity is indicative of past or present caries activity; a filling is essentially a record of previous caries experience. Between the time of restoration or cessation of active caries and the date of tooth extraction, fluorine may have been acquired, that is, posteruptively.[15, 16] It is important to note, therefore, that our designation as "carious" actually refers to teeth which had caries at a prior undetermined period and may or may not have been actively carious at the time of extraction.

The content of ash was determined as a routine part of the fluorine determination and is recorded in two of the tables. The ash data are consistent for all teeth, both sound and carious. The possibility that fluorine in the ash might be more revealing with regard to differences in fluorine content than fluorine in the unashed sample was given some consideration, but comparisons of fluorine values for ashed samples are no different from comparisons of fluorine in the unashed dentin or enamel. The major attention is given to fluorine in the unashed samples.

Fluorine was determined in dentin and enamel according to Armstrong's modification[17, 18] of the Willard and Winter titration technique.[19] Individual teeth was first cleaned and the crowns separated from the roots. All carious material was removed with dental burs. The crowns were pulverized to pass a 60-mesh sieve, extracted in a Soxhlet eight hours with ether and eight hours with alcohol, and dentin and enamel were separated by the flotation process of Manly and Hodge.[20]

The enamel and dentin samples were dried at 100° C. for one hour, and a sample for fluorine analysis was weighed into a tared platinum crucible. The sample was ashed at 550° C. for three hours and the weight of ash determined. The ash was transferred to a 50 c.c. pyrex Claisen distilling flask containing a glass bead, and 10 c.c. of 60 per cent $HClO_4$ were added with about 10 c.c. of water used for washing the ash from the crucible. Steam distillation was begun when the temperature in the distilling flask reached 138 to 140° C. and was continued until 150 c.c. of distillate were collected in a receiving flask containing 5 c.c. N/10 NaOH. The distillate was evaporated almost to dryness in a platinum dish, then made acid with N/10 HCl, and transferred to a 10 c.c. volumetric flask. Three or four aliquots, consisting of $^1/_5$ or $^1/_{10}$ of the total, were titrated with standard thorium nitrate, after first buffering with one drop of the usual trichloracetate buffer and adding one drop of sodium alizarinsulfonate indicator. The mean of three or four aliquot titrations was used for the final calculations. In the majority of the analyses the quantity of determined fluorine was not less than 25.0 μg and rarely exceeded 100 to 150 micrograms. This necessitated starting with an analytical sample of enamel or dentin of the order of 0.2 to 0.5 Gm., which in most instances made it necessary to pool enamel and dentin from several teeth. Teeth of a similar type were pooled in all but a very few instances. Some idea of the accuracy of the method may be gained

from the analytical and recovery data shown in Table II. Twenty-five and fifty micrograms of fluorine as sodium fluoride were added to dentin and enamel samples previously analyzed for fluorine. The micrograms and per cent of fluorine recovered after the mean per cent of fluorine present in the sample was deducted, are recorded in Table II.

TABLE II

FLUORINE ANALYSES OF HUMAN DENTIN AND ENAMEL—RECOVERY OF ADDED FLUORINE

	ANALYSIS				
	1	2	3	4	MEAN
	Sample A (Sound Dentin)				
Per cent fluorine	.0228	.0230	.0231	--	.0230
Fluorine added μg	25.0	25.0	50.0	50.0	--
Fluorine recovered μg	27.7	29.0	52.7	42.6	--
Fluorine recovered %	110.8	116.0	105.4	85.2	104.4
	Sample B (Carious Dentin)				
Per cent fluorine	.0195	.0198	.0169	.0143	.0176
Fluorine added μg	25.0	25.0	25.0	50.0	--
Fluorine recovered μg	26.3	21.6	26.2	48.8	--
Fluorine recovered %	105.2	86.4	95.4	95.2	95.6
	Sample C (Carious Enamel)				
Per cent fluorine	.0083	.0081	.0080	.0094	.0085
Fluorine added μg	25.0	50.0	50.0	--	--
Fluorine recovered μg	20.5	44.2	47.6	--	--
Fluorine recovered %	82.0	88.4	95.2	--	88.5
	Sample D (Sound Enamel)				
Per cent fluorine	.0107	.0124	.0100	.0122	.0113
Fluorine added μg	25.0	50.0	50.0	--	--
Fluorine recovered μg	24.7	53.0	44.7	--	--
Fluorine recovered %	98.8	106.0	89.4	--	98.0
	Sample E (Sound Enamel)				
Per cent fluorine	.0120	.0114	--	--	.0117
Fluorine added μg	25.0	25.0	--	--	--
Fluorine recovered μg	24.1	25.8	--	--	--
Fluorine recovered %	96.4	103.2	--	--	99.8

The error in the fluorine determination for dentin and enamel according to the data in Table II is ±5.0 to 10.0 per cent.

RESULTS

Any comparison of sound and carious teeth which is made between different individuals for fluorine differences is likely to introduce errors due to variations in the individual's fluorine exposures. Dietary fluorine which is influenced almost exclusively by the drinking-water fluorine[22] may be expected to affect the fluorine in teeth, particularly during pre-eruptive tooth life, and may also increase dental tissue fluorine posteruptively.[15, 16, 21] In Table III, this uncontrolled variation in fluorine exposures of sound and carious groups of teeth is disregarded and all the analytical data are averaged. In one group, there were fourteen individuals who were continuous residents of McDuffie County, Ga., where the caries prevalence is somewhat low and there is no evidence of an unusual fluoride exposure. The forty-four individuals listed as residing in northcentral Illinois constitute a variable group whose residence history was not established and remains quite uncertain. Although the teeth

TABLE III

RESULTS OF ANALYSIS OF SOUND AND CARIOUS TEETH. PER CENT FLUORINE, ASH, AND FLUORINE IN ASH IN ENAMEL AND DENTIN

		DENTIN								ENAMEL							
		SOUND TEETH				CARIOUS TEETH				SOUND TEETH				CARIOUS TEETH			
NUMBER OF SOUND TEETH	NUMBER OF CARIOUS TEETH	NUMBER OF ANALYSES	ASH (%)	F (%)	F IN ASH (%)	NUMBER OF ANALYSES	ASH (%)	F (%)	F IN ASH (%)	NUMBER OF ANALYSES	ASH (%)	F (%)	F IN ASH (%)	NUMBER OF ANALYSES	ASH (%)	F (%)	F IN ASH (%)
14 Individuals Who Were Continuous Residents of McDuffie City, Ga.—Nonfluoride Area																	
16	13	21	76.4	.0216	.0283	19	77.1	.0186	.0242	6	94.7	.0086	.0091	10	94.9	.0097	.0102
44 Individuals Residing in Northcentral Ill.—Moderate Fluoride Area																	
22	33	5	80.2	.0300	.0373	8	79.6	.0210	.0264	6	96.4	.0103	.0106	7	95.5	.0093	.0097
33 Individuals From Scattered Areas With No Indication of Fluorine Exposure																	
224	202	124	80.0	.0240	.0302	160	79.8	.0227	.0285	120	95.9	.0102	.0106	125	95.7	.0098	.0102
Total 262	248	150	--	--	--	187	--	--	--	132	--	--	--	142	--	--	--
Mean*		--	79.8	.0241	.0302	--	79.7	.0225	.0282	--	95.9	.0102	.0106	--	95.7	.0098	.0102
P.E. mean±		--	.3	.0010	.0012	--	.2	.0007	.0009	--	.1	.0004	.0004		.1	.0003	.0003
		Sound vs. Carious – differences								Sound vs. Carious – differences							
Ash				0.1 ± 0.4								0.2 ± 0.1					
Fluorine				0.0016 ± 0.0012								0.0004 ± 0.0005					
Fluorine in Ash				0.0020 ± 0.0015								0.0004 ± 0.0005					

*In calculation of this mean and its probable error, the result for each individual of the group of 33 individuals was used as a separate item, and the means of the other two groups constituted two other items. Among the group of 33 individuals, from 1 to 14 analyses per individual were averaged to obtain the individual items.

from these individuals were collected in a geographical area which has a history of moderate fluoride waters (1.5 to 2.5 ppm F),[2] no elevation in enamel and dentin fluorine was disclosed by analysis. The data for thirty-three individuals were studied separately. These individuals constitute a random group representing individuals whose extractions were performed mostly in Washington, D. C. Their residence histories, so far as they could be obtained, varied greatly. The differences among the means of all the data for ash, fluorine, and fluorine in ash, shown in Table III, have no statistical significance. These mean values were calculated from 132, 142, 150, and 187 individual analyses, representing 262 sound and 248 carious teeth from ninety-one individuals. The error due to nonrandom sampling differences (caused by variable fluorine exposures) in these group data may not be too significant in consideration of the relatively large number of individuals and teeth represented in the data. It should be noted also that none of these teeth had a pre-eruptive fluorine exposure large enough to produce mottled enamel. The extent of the variability in the data for different individuals is evident in the figures shown in Table IV and may be explained as mentioned previously by the sensitivity of dental tissues to dietary fluorine.

TABLE IV

FLUORINE CONTENT OF DENTIN AND ENAMEL OF SOUND AND CARIOUS TEETH, COMPARISONS MADE ON THE SAME INDIVIDUAL

		DENTIN			ENAMEL			RATIO	
								DENTIN VS. ENAMEL	
INDIVIDUAL	AGE (YR.)	SOUND (% F)	CARIOUS (% F)	DIFFERENCE (% F)	SOUND (% F)	CARIOUS (% F)	DIFFERENCE (% F)	SOUND TEETH	CARIOUS TEETH
1	35	.0156	.0254	+.0098	.0070	.0109	+.0039	2.2	2.3
2	43	.0223	.0163	−.0060	.0073	.0054	−.0019	3.0	3.0
5	53	.0312	.0327	+.0015	--	--	--	--	--
6	35	.0133	.0119	−.0014	--	--	--	--	--
7	40	.0210	.0183	−.0027	.0096	.0068	−.0028	2.2	2.7
8	46	.0202	.0206	+.0004	.0104	.0087	−.0017	1.9	2.4
9	26	.0319	.0294	−.0025	.0173	.0154	−.0019	1.8	1.9
10	23	.0246	.0270	+.0024	.0152	.0140	−.0012	1.6	1.9
11	45	.0205	.0169	−.0036	.0100	.0083	−.0017	2.1	2.0
12	--	.0256	.0216	−.0040	.0154	.0139	−.0015	1.7	1.6
17	--	.0175	.0235	+.0060	.0079	.0092	+.0013	2.2	2.6
20	60	.0191	.0205	+.0014	.0076	.0080	+.0004	2.5	2.6
21	39	.0219	.0201	−.0018	.0087	.0068	−.0019	2.5	3.0
23	56	.0156	.0194	+.0038	.0078	.0082	+.0004	2.0	2.4
24	--	.0204	.0210	+.0006	.0074	.0096	+.0022	2.8	2.2
25	42	.0313	.0311	−.0002	.0100	.0102	+.0002	3.1	3.1
26	51	.0240	.0222	−.0018	.0110	.0124	+.0014	2.2	1.8
28	--	.0168	.0197	+.0029	.0067	.0078	+.0011	2.5	2.5
31	47	.0136	.0177	+.0041	--	--	--	--	--
	Mean	.0214	.0219	+.0005*	.0100	.0097	−.0003*	2.3	2.4
	P.E.$_m$	±.0008	±.0008	±.0011†	±.0005	±.0005	±.0007†	±.07	±.07

*Difference between means.

†P. E. difference.

Statistical analysis of differences according to Student[23]:

	FLUORINE SOUND VS. CARIOUS DENTIN	FLUORINE SOUND VS. CARIOUS ENAMEL
Mean difference	+.00005	−.00004
Standard deviation of difference	.00008	.00004
t value	.620	1.071
probability	.272	.151

Among the thirty-three individuals studied separately, there are nineteen who provided both sound and carious dentin and sixteen represented with both sound and carious enamel. A comparison of the fluorine in sound and carious dentin and enamel from these individuals is shown in Table IV. The statistical comparison of the sound and carious "means" in Table IV gains in significance because of the presence of teeth from the same individuals in both sound and carious categories. The mean difference between the amount of fluorine in sound and in carious dentin is +.0005 ±.0011 per cent. Between sound and carious enamel the fluorine difference is –.0003 ±.0007 per cent. Neither of these differences is statistically significant. When the individual differences were treated statistically by Student's method,[23] the probability factors, .272 for sound versus carious dentin and .151 for sound versus carious enamel, were obtained (Table IV). These factors give no indication that the differences are significant. Only a probability factor of 0.05 or less justifies a significant interpretation (the odds are 1 to 20 or more that the result is not a chance happening). Therefore, in this comparison of sound and carious data (eighteen dentin and sixteen enamel) from the same individuals, there is no indication that differences in fluorine content could be related to the carious or noncarious status of the tooth.

Different developmental and calcification characteristics of the various functional types of teeth (as well as variations in fluorine exposure) might introduce variations in the amount of fluorine acquired by the teeth, particularly as they develop and calcify. An attempt to eliminate this developmental variable is made in the analysis of the data shown in Table V where the data compare

TABLE V

FLUORINE ANALYSES OF SOUND AND CARIOUS TEETH FROM THE SAME INDIVIDUAL (COMPARISONS MADE BETWEEN TEETH OF SIMILAR TYPE)

TYPE	NUMBER OF COMPARISONS	DENTIN		ENAMEL	
		SOUND (% F)	CARIOUS (% F)	SOUND (% F)	CARIOUS (% F)
A	10	.0197	.0210	.0085	.0096
B	6	.0232	.0232	.0113	.0113
C	2	.0200	.0133	.0101	.0082
Mean		.0209	.0214	.0095	.0099
P. E. mean		±.0010	±.0010	±.0006	±.0007
Difference		.0005		.0004	
P. E. difference		±.0014		±.0009	

Type A, First incisors, second incisors, canines; Type B, First premolars, second premolars; Type C, Second molars.

carious and sound teeth of similar type from the same individual. For example, Individual 1 had both sound and carious first incisors, or second incisors, or canines represented in the data. The differences (and their probable errors) in the fluorine found in the sound and carious enamel and dentin are tabulated (Table V). These differences have no statistical significance; the probable error of each calculated difference is greater than the difference itself.

A study of the results with respect to teeth having different developmental characteristics was made also by means of the data shown in Tables VI and VII.

Here again, the uncontrolled, unknown variation in the individual's exposure to fluorine may have influenced the quantity of fluorine deposited, particularly during the formative period. The mean differences tested statistically according to the method of Student[23] gave probability factors permitting no interpretation of significance (Table VI).

TABLE VI
COMPARISON OF FLUORINE IN DIFFERENT TYPES OF TEETH FROM THE SAME INDIVIDUAL

COMPARISONS	NUMBER OF INDIVIDUAL COMPARISONS	MEAN DIFFERENCE IN FLUORINE (%)	PROBABILITY FACTOR*
Canines vs. incisors			
Sound dentin	14	−.00012	.127
Sound enamel	13	−.00001	.461
Premolars vs. combined incisors and canines			
Sound dentin	10	−.00015	.073
Sound enamel	12	+.00009	.060
2nd molars vs. premolars			
Carious dentin	13	+.00002	.577
Carious enamel	12	−.00007	.073

*According to Student.[23]

All of the data have been utilized in the preparation of Table VII to study fluorine in the different anatomic types of teeth and in sound and carious teeth. Statistically the differences in data for sound and carious teeth in this table have no significance. The different teeth (combining all the sound and carious) vary in fluorine content between .0230 per cent and .0247 per cent in the dentin and between .0086 per cent and .0120 per cent in the enamel.

While the variation in fluorine in individual teeth is indicated to some extent by the data presented in Table VII, some further idea as to the variations

TABLE VII
FLUORINE IN SOUND AND CARIOUS TEETH CLASSIFIED ACCORDING TO TYPE

	SOUND			CARIOUS			SOUND AND CARIOUS		SOUND AND CARIOUS	
	NUMBER OF SAMPLES	MEAN	P.E.	NUMBER OF SAMPLES	MEAN	P.E.	NUMBER OF SAMPLES	MEAN	P.E.	DIFFERENCE	P.E.
Fluorine in Dentin (Per Cent)											
Incisors	44	.0252	±.0009	28	.0231	±.0010	72	.0240	±.0007	.0021	±.0013
Canines	24	.0241	±.0011	19	.0219	±.0012	43	.0231	±.0008	.0022	±.0016
1st premolars	20	.0252	±.0011	18	.0212	±.0009	38	.0233	±.0008	.0040	±.0014
2nd premolars	19	.0276	±.0010	20	.0216	±.0009	39	.0245	±.0007	.0060	±.0013*
1st molars	10	.0265	±.0025	19	.0215	±.0010	29	.0232	±.0011	.0050	±.0027
2nd molars	13	.0245	±.0016	45	.0225	±.0008	58	.0230	±.0007	.0020	±.0018
3rd molars	18	.0276	±.0018	18	.0221	±.0011	34	.0247	±.0011	.0055	±.0021
Fluorine in Enamel (Per Cent)											
Incisors	22	.0118	±.0005	22	.0101	±.0005	44	.0110	±.0004	.0017	±.0007
Canines	21	.0093	±.0004	13	.0094	±.0004	34	.0093	±.0003	.0001	±.0005
1st premolars	15	.0108	±.0005	16	.0089	±.0005	31	.0098	±.0004	.0019	±.0007
2nd premolars	20	.0101	±.0005	17	.0115	±.0007	37	.0107	±.0004	.0014	±.0008
1st molars	9	.0074	±.0004	16	.0093	±.0005	25	.0086	±.0004	.0019	±.0006*
2nd molars	12	.0089	±.0005	35	.0108	±.0004	47	.0104	±.0004	.0010	±.0006
3rd molars	15	.0116	±.0006	16	.0124	±.0005	31	.0120	±.0004	.0008	±.0008

*Significant statistical differences are in excess of three times the probable error of the difference.

which may occur among individual teeth in the same mouth may be gained by inspection of the data shown in Table VIII. In this table are presented the detailed results of analyses of all the teeth available from three individuals. The differences in the fluorine content of individual teeth from the same dentition may be considerable, as the data show for Individual 13, particularly. All the teeth obtained from this individual were sound; the enamel fluorine varied from 0.0053 per cent to 0.0123 per cent, and the dentin varied from 0.0166 per cent to 0.0327 per cent fluorine. As a rule, the data for individual teeth were more uniform than that shown for Individual 13.

TABLE VIII

FLUORINE IN DENTIN AND ENAMEL (ANALYTICAL DATA AS OBTAINED FOR THREE INDIVIDUALS)

INDIVIDUAL 13			INDIVIDUAL 3			INDIVIDUAL 10		
TEETH EXTRACTED IN WASHINGTON, D. C.; AGE AND RESIDENCE UNKNOWN			46-YEAR-OLD MAN; CONTINUOUS RESIDENCE IN MCDUFFIE COUNTY, GA.			23-YEAR-OLD MAN; ENTIRE LIFE IN WASHINGTON, D. C. AREA		
	SOUND			SOUND			CARIOUS	
TOOTH NUMBER*	DENTIN (% F)	ENAMEL (% F)	TOOTH NUMBER*	DENTIN (% F)	ENAMEL (% F)	TOOTH NUMBER*	DENTIN (% F)	ENAMEL (% F)
1, 16	.0166	.0064	16	.0397	.0101	2	.0322	.0159
2, 15	.0240	.0114	3, 14	.0405	.0058	15	.0303	.0147
3, 14	.0228	.0053	4, 13, 20, 29	.0352	.0070	13	.0284	.0129
4, 13	.0270	.0103	5, 12, 21, 28	.0313	.0064	14	.0253	.0099
5, 12	.0284	.0120	6, 11, 22, 27	.0342	.0100	1, 3	.0248	.0132
6, 11	.0277	.0114	7, 10, 23	.0478	.0072	12	.0248	.0116
7, 10	.0293	.0117	8, 9, 24, 25	.0473	.0068	6, 11†	.0220	.0102
8, 9	.0308	.0114	32, 17	.0446	.0095	32	.0337	.0146
32, 17	.0233	.0100	31, 18	.0313	.0069	18	.0184	.0128
31, 18	.0217	.0064	30, 19	.0480	.0084	27, 22	.0249	.0127
30, 19	.0186	.0098						
29, 20	.0274	.0123						
28, 21	.0327	.0120						
26, 23	.0236	.0094						
Mean	.0253	.0100	Mean	.0400	.0078	Mean	.0265	.0129

*In most instances two or more teeth of similar type were pooled in order to obtain a quantity of analytical sample sufficient for one complete analysis. Teeth are numbered according to following formula: Upper right third molar (Number 1) to upper left third molar (Number 16); lower left third molar (Number 17) to lower right third molar.
†Sound.

DISCUSSION

One of the purposes of this study was to attempt to explain the role of fluorine as a caries-preventive element. One hypothesis receiving reasonably sound support ascribes the anticaries effects of fluoride domestic waters[2] and the effect of topically applied fluoride[24, 25] to the acquisition of an optimal quantity of fluorine by dental tissue. This hypothesis has been supported by the widely accepted but limited data of Armstrong and Brekhus[7] which, as quoted previously, show significant differences in the fluorine content of the enamel of sound versus carious teeth of three individuals. Other group-data presented by these same investigators reflect the same enamel differences, although no differences were observed in the dentin.

In the light of more extensive analytical data there seems considerable reason to believe that a difference in fluorine content of sound and carious enamel (and dentin) is not a general rule among most individual dentitions. The sound and carious enamel in the teeth of all of the sixteen individuals showed

no differences, and in all of the nineteen individuals there were no consistent differences in the sound and carious dentin. Therefore, it does not appear that the caries history of one tooth as compared with another tooth in the same dentition was related to its fluorine content.

Analysis of the data in relation to variations in the fluorine content of different functional or anatomic types of teeth has brought to light no significant variations in either dentin or enamel. As in the comparisons of carious and sound teeth, it is interesting to note that the statistical results for both dentin and enamel indicate a similar pattern of uniformity. This relation is substantiated in the dentin to enamel ratios shown in Table IV. The dentin seems to contain about 2.0 to 2.5 times as much fluorine as the enamel. In this connection, previous analyses of rats' molar and incisor teeth may be noted in which the dentin to enamel fluorine ratio was of the order of 2.5 to 3.0 for the incisors; and where fluorine had been acquired (even in varying quantities) posteruptively by the rats' molars, there was uniformly 3.5 to 4.0 times as much fluorine in the dentin as in the enamel.[16] This relatively consistent relation of fluorine in dentin to fluorine in enamel in the case of rats' teeth was ascribed to an orderly systemic retention of fluorine by both dentin and enamel, which seemingly was unaffected by "oral surface absorption of fluorine." Human teeth-fluorine data also give good evidence of a relatively consistent ratio of fluorine in the dentin to fluorine in the enamel. This would not be the case in the carious tooth if, as the data of Armstrong and Brekhus[7] would indicate, there were less fluorine in the enamel but not in the dentin of the carious tooth. The weight of the evidence according to our data suggests, therefore, that all the teeth in a dentition generally respond with considerable uniformity to dietary-fluorine exposure, and fluorine in the dentin and enamel will be in a relatively uniform ratio. Variations among teeth appear to have no relation to the anatomic type or subsequent caries history.

While teeth studied individually in a single dentition may show no relation, therefore, between fluorine content and individual caries experience, such a finding is not contrary to the suggestion that the total caries experience of an individual may be greatly benefited by the presence of an over-all optimum quantity of fluorine in the dentin and enamel of all the teeth. It may be possible, therefore, to attribute the decrease in dental caries experience among individuals residing in areas of domestic fluoride waters[2] to an over-all increase in fluorine in the dentin and enamel of all the teeth. In this way a critical concentration of fluorine in the dentin and enamel of the entire dentition may be expected to improve the total dental caries experience of the individual. In the future, our studies will attempt to relate fluorine in dentin and enamel to fluoride-domestic-water exposure, and these results may throw light on the relation of fluorine in dentin and enamel to the communal dental caries index. At the same time, additional data will be available to study sound and carious teeth as well as mottled teeth which have been exposed to domestic fluoride waters.

SUMMARY

The fluorine content of human dentin and enamel has been studied by the analyses of teeth obtained from thirty-three individuals (studied separately)

and from two groups of fourteen and forty-four individuals each. The crowns of 262 sound and 248 carious teeth were separated into dentin and enamel and a little over 600 fluorine determinations made on pooled or individual dentin and enamel samples. None of the teeth showed macroscopic fluorosis and, with the exception of the group of forty-four individuals, there was no reason to suspect any unusual fluoride exposure. The ash in enamel averaged 95.8 per cent; in dentin, 79.8 per cent; and no differences were apparent between sound and carious teeth. The fluorine in enamel averaged .0100 per cent and in dentin .0233 per cent, and the differences between the enamel and the dentin of sound and carious teeth were not significant. Fluorine in the ash of enamel averaged .0104 per cent and in the ash of dentin, .0292 per cent. Differences between results for sound and carious teeth were not significant. A study of the teeth according to anatomic or functional type also did not reveal any significant differences in fluorine content.

An evaluation of the analytical data at present suggests that fluorine data for the individual teeth of a single dentition cannot be expected, as a general rule, to show consistently less fluorine in the enamel of the carious teeth than in the enamel of the sound teeth. In some individuals, fluorine in the enamel of the sound teeth alone may differ as much as the fluorine in enamel of sound versus carious teeth.

There is reason to believe that an unusual dietary (particularly water-fluoride) exposure during formative tooth life may increase the fluorine content of the entire dentition, including both dentin and enamel. An increase in fluorine in all the teeth may then account for an over-all reduction in the dental caries experience. Under this circumstance it may be said that an optimum quantity of fluorine in the dentin and the enamel of the entire dentition is associated with a reduced incidence of dental caries. At the present time, data are not available to demonstrate this later hypothesis, but such an investigation is under way.

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THE INCIDENCE OF DENTAL CARIES AMONG FRESHMAN STUDENTS AT OREGON STATE COLLEGE*

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FROM epidemiological studies on dental caries conducted among the same age groups of people living in different geographic areas of the United States, it has been found that there is a difference in the incidence of the disease among them. As an explanation for this variability in caries incidence of population groups, the effect of several intrinsic and extrinsic factors on the incidence of dental decay has been analyzed.

In a recent report[1] on caries prevalence (decayed and missing teeth) among draftees from all the states, it was pointed out that the State of Oregon had the fifth highest position among the forty-eight states. In view of this fact, and because there were no detailed data available concerning the dental condition of the people of the state of Oregon, this study was undertaken in order to find out the incidence of dental caries among a selected group of people and also some of the possible etiological factors which influence it. It was hoped that the findings of this survey would serve as a basis for further investigation in selected geographic areas of the State of Oregon.

MATERIAL AND PROCEDURE

The subjects of this investigation were freshman students of Oregon State College who, according to the registrar's records, were residents of the State and did not serve in the Armed Services during the war. The purpose of eliminating nonresidents and veterans was to have as homogeneous a group as possible.

The data collected included a dental examination, saliva analyses, and the taking of a nutrition history.† This paper deals only with the dental examination and findings. The following items were recorded on the dental examination form: (1) Number of teeth which had been filled; (2) number of missing teeth; (3) number of teeth indicated for extraction because of extensive caries; (4) number of cavities indicated for filling detected by explorer, and (5) number of cavities indicated for filling detected by radiographs.

All students were examined by one of us (D. H.), and this fact adds more uniformity to the collected material, as the method of examination was the same for all the subjects.

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†Data to be published.

The dental examination was done by means of mouth mirror and explorer in good, natural light combined with a dental spotlight. Posterior bite-wing radiographs were taken of each student. An illuminator and magnifying glass were employed for viewing the radiographs. Pits and fissures were listed as carious if the explorer caught and only when a carious lesion was actually found by careful examination. Observations were not made for third molars. All missing teeth were assumed to have been lost because of extensive caries, except in a few cases where the subject upon questioning stated other reasons, for example, lost by accident or congenitally missing. In these instances, the missing teeth were not listed.

The dental caries experience was measured by counting the number of filled teeth, missing teeth because of caries, teeth indicated for extraction, and teeth with untreated dental caries indicated for filling. The term "DMF" was used to designate past and present caries experience for teeth and tooth surfaces.

FINDINGS

The sex and age distributions of the 582 freshman students examined are given in Table I. The number of women was higher than men in all age groups. Most of the students fell within the 18-year-old group. In the age group 17 and under (17–) only one student was sixteen, while in the age group 20 and over (20–), most of the students were 20 years old with only a few above this age. All of the counties of the State of Oregon except Grant and Wallowa were represented in the sample. Counties such as Multnomah, Marion, Benton, and Linn, where larger cities and towns are located, had a greater number of students attending Oregon State College. Four of the 582 students were found caries-free.

The data given in Table II provide information as to the dental caries experience (DMF) in terms of teeth and tooth surfaces per student. As it can be seen, the average number of DMF teeth and tooth surfaces increases with the

TABLE I

NUMBER OF FRESHMAN STUDENTS AT OREGON STATE COLLEGE GIVEN DENTAL EXAMINATION, BY SPECIFIED AGE AND SEX GROUPS

FRESHMAN STUDENTS	AGE IN YEARS (LAST BIRTHDAY)				
	17–	18	19	20+	ALL AGES
No. of men	13	172	39	11	235
No. of women	21	214	82	30	347
No. of both sexes	34	386	121	41	582

TABLE II

NUMBER OF DMF PERMANENT TEETH AND TOOTH SURFACES BY SPECIFIED AGE GROUPS OF 582 FRESHMAN STUDENTS AT OREGON STATE COLLEGE. RATES ARE EXPRESSED PER STUDENT

PERMANENT TEETH	AGE IN YEARS (LAST BIRTHDAY)				
	17–	18	19	20+	ALL AGES
No. of DMF teeth	13.00	14.28	14.50	14.68	14.12
No. of DMF surfaces	28.71	33.51	36.02	35.37	33.40

chronological age of the student, except in the case of the 20-year-old group. This was presumably due to the small number of students of that age examined.

The average number of DMF teeth of the 17-year-old students was 13.0 and increased to 14.7 at the age of 20 years. The number of tooth surfaces, which gives a more accurate picture of caries experience, increased from an average of 28.7 at the age of 17 to around 36.0 at the age of 20 years.

In order to be able to compare these data with the findings of surveys conducted in other parts of the country, it was necessary to eliminate the carious lesions detected by means of bite-wing radiographs which have contributed to the DMF teeth rates reported. Table III shows the numbers of DMF teeth, exclusive of carious lesions detected by radiographs, of freshman students at Oregon State College as compared with high school students from Hagerstown, Md., San Francisco, and New York City.[2] On this basis, the 17-year-old student at Oregon State College had an average of 12.2 DMF teeth while his counterpart in Hagerstown, San Francisco, and New York City had an average of approximately 8.0 DMF teeth. A similar difference was also observed in the other age groups.

TABLE III

COMPARISON OF AVERAGE NUMBER OF DMF TEETH PER PERSON BY SPECIFIED AGE GROUPS IN OREGON,* HAGERSTOWN, MD., SAN FRANCISCO, AND NEW YORK CITY

LOCALITY	AGE IN YEARS (LAST BIRTHDAY)			
	17	18	19	20+
Oregon	12.24	13.51	13.93	14.24
Hagerstown, Md.	7.73	8.72	9.25	--
San Francisco	7.7	8.3	--	--
New York City	7.99	8.69	6.94	--

*To make the above comparison, it was necessary to determine the DMF teeth exclusive of the carious lesions detected by posterior bite-wing radiographs.

There are reports in the literature[3, 4] showing that girls have higher dental caries experience rates than boys of the same chronological age. This is also demonstrated in Table IV. The 582 freshman students were divided according to sex and age distribution, and their DMF teeth and tooth surfaces were calculated. It is evident from this table that in all age groups the dental caries experience of the women was higher than that of men. This does not mean that females are more susceptible to the disease than males. It has been shown[5] that this phenomenon is due to the fact that the teeth of girls erupt earlier than those of boys and therefore they are exposed longer to the factors which influence the occurrence of the disease.

TABLE IV

NUMBER OF DMF PERMANENT TEETH AND TOOTH SURFACES BY SPECIFIED AGE AND SEX GROUPS OF 582 FRESHMEN STUDENTS AT OREGON STATE COLLEGE; RATES ARE EXPRESSED PER STUDENT

PERMANENT TEETH	MEN					WOMEN				
	AGE IN YEARS (LAST BIRTHDAY)				ALL AGES	AGE IN YEARS (LAST BIRTHDAY)				ALL AGES
	17–	18	19	20+		17–	18	19	20+	
DMF teeth	13.00	14.07	13.51	13.82	13.60	13.00	14.45	14.96	15.00	14.35
DMF surfaces	28.23	32.88	33.05	32.36	31.63	29.00	34.01	37.43	36.47	34.23

Table V gives a picture of the past and present caries experience of the subjects by specified age and sex groups. Although women experienced higher DMF teeth and tooth surfaces than men (as it was shown in Table IV), their

TABLE V

NUMBER OF FILLED TEETH, EXTRACTED TEETH, TEETH REQUIRING EXTRACTION, AND CAVITIES REQUIRING FILLING OF 582 FRESHMAN STUDENTS OF OREGON STATE COLLEGE. RATES ARE EXPRESSED PER STUDENT BY SPECIFIED AGE AND SEX GROUPS

PERMANENT TEETH	MEN: AGE IN YEARS (LAST BIRTHDAY) 17–	18	19	20+	ALL AGES	WOMEN: AGE IN YEARS (LAST BIRTHDAY) 17–	18	19	20+	ALL AGES
Filled	8.77	10.19	10.56	9.18	9.68	10.57	11.71	12.05	11.73	11.52
Extracted	0.46	0.66	0.72	1.82	0.92	0.43	0.57	1.18	1.23	0.85
Requiring extraction	--	0.10	0.05	0.18	0.08	0.10	0.03	0.02	0.10	0.06
Cavities requiring filling	7.46	6.72	5.13	5.18	6.12	4.71	5.11	4.49	4.37	4.67

mouths, nevertheless, were in better condition. In general, they had fewer missing teeth, fewer teeth requiring extraction, and fewer open cavities requiring filling. This is rather difficult to explain on the basis that women are more conscious of the significance of dental health. Presumably, it is because women take better care of their teeth for esthetic reasons. Klein and Palmer,[6] after studying the dental status of school children of forty communities in New Jersey, concluded that the economic status of the community in which the children lived did not affect the incidence of dental caries, but that, on the other hand, it influenced the amount of dental service given to the individual. The more prosperous the community was, the greater the amount of dental care received. This means that more teeth are saved by fillings and less are neglected to such an extent as to require extraction. As it has been shown in Table III, the high school children in Hagerstown, Md., had a lower rate of DMF teeth than did freshman

TABLE VI

NUMBER OF MISSING TEETH AND TEETH REQUIRING EXTRACTIONS AMONG 582 FRESHMAN STUDENTS AT OREGON STATE COLLEGE AND 1841 HIGH SCHOOL CHILDREN AT HAGERSTOWN, MD. RATES ARE EXPRESSED PER SUBJECT BY SPECIFIED AGE GROUPS

LOCALITY	PERMANENT TEETH	AGE IN YEARS (LAST BIRTHDAY) 17	18	19	20+
Oregon	Missing	0.45	0.62	0.95	1.53
	Extraction ind.	0.05	0.07	0.04	0.14
Hagerstown, Md.	Missing	1.27	1.80	2.78	--
	Extraction ind.	0.15	0.21	0.16	--

students of Oregon State College. On the other hand, the students at Oregon State College showed fewer teeth missing and also fewer teeth requiring extraction than their counterparts in Hagerstown, Md.[7] (Table VI). This difference can probably be attributed to the better economic status of the families from

TABLE VII

COMPARISON OF CAVITIES REQUIRING FILLING DETECTED BY MEANS OF CLINICAL AND X-RAY EXAMINATIONS OF 582 FRESHMAN STUDENTS AT OREGON STATE COLLEGE; RATES ARE EXPRESSED PER STUDENT

TOTAL NUMBER	NUMBER DETECTED BY EXPLORER	NUMBER DETECTED BY RADIOGRAPH	PER CENT DETECTED BY RADIOGRAPH
5.50	3.83	1.67	30.36

which the college students come. At the same time, such factors as better understanding of the importance of dental health and better distribution of dentists throughout the state may have influenced it.

From previous reports,[8-10] the importance of including radiographs in the dental examination as a diagnostic tool for the detection of carious lesions has been demonstrated. Table VII shows the total number of cavities per student requiring filling as well as the number of cavities which were detected by explorer and by the taking of posterior bite-wing radiographs. On the average, there are 5.5 cavities per student requiring filling. Of these, 3.8 were found by using the explorer alone, and 1.7 by taking posterior bite-wing radiographs. In other words, 30.4 per cent of the cavities requiring filling would have been missed if posterior bite-wing radiographs had not been taken. This is very significant, although the number of cavities which could be missed if bite-wing radiographs are not included in the examination may fluctuate considerably, depending upon the ability of the examiner as well as the time devoted for each examination.

One of the aims of this study was an attempt to find variations in the caries experience of students coming from the different geographic regions of the State with the thought of further local investigation. For this purpose the State of Oregon was divided into six geographic areas: Coast region, Willamette Valley, Blue Mountains, Southern Oregon, Columbia Basin, and Central Oregon. Statistical treatment of the data* revealed that the students from the Coast region had the highest and that those from the Central Oregon region had the lowest dental caries experience. Based on this information, plans have been made for local studies of the factors which might be responsible for the high or low rate of attack.

SUMMARY

Freshman students at Oregon State College were given a dental examination, including posterior bite-wing radiographs, and the following items of interest were noted:

The dental caries experience of the 582 students, as compared with subjects of the same age groups examined at various geographic areas of the country, was considerably higher.

Female students showed a higher rate of DMF teeth and tooth surfaces than male students.

The teeth of female students had received greater dental care.

In general, the students at Oregon State College, although they had a high caries experience, showed fewer teeth missing and fewer teeth requiring extraction when compared with subjects from other studies in which lower rates of caries experience were reported. The better financial status of the families from which the students come, as well as other factors, is considered as an explanation.

The value of bite-wing radiographs as a diagnostic tool for the detection of carious lesions was demonstrated.

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Geographic division of the State of Oregon into six areas revealed that the students from the Coast Region showed the highest incidence of dental caries and that those coming from the Central Oregon region showed the lowest incidence of dental caries. However, from these data it cannot be assumed that this applies to the general populations of the two regions.

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FURTHER STUDIES ON THE ASSOCIATION BETWEEN THE PRODUCTS OF PROTEIN PUTREFACTION AND DENTAL CARIES ACTIVITY

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THE existence of an antithetic relationship between dental caries and periodontal disease has been postulated by a number of investigators. Berg and co-workers[1] observed that "It is a common clinical finding that chronic periodontitis and dental caries are generally antagonistic." Broderick,[2-4] in a number of papers, stressed the presence of an antagonism between dental caries and periodontal disease. In support of this contention he quoted from the works of Price, Pickerill, Raper, Bibby, and Prinz, and attributed the antagonism to a generalized condition of acidosis and alkalosis.[4] In his studies he found that dental caries predominated in the presence of acidosis and that periodontal disease predominated when alkalosis was present. While the systemic aspect of this explanation is still open to question, the majority of the investigations dealing with this subject indicate that biochemically, at least, dental caries results primarily from a degradation of carbohydrates by acidogenic organisms, a process characterized by the production of acids, whereas periodontal disease is the result of a proteolytic action in which ammonia is produced and which is alkaline in nature.[5]

In a previous report, Dreizen and Spies[6] found that relatively dilute solutions of either indole or indole-3-acetic acid, both produced during the bacterial decomposition of tryptophane, are capable of interfering with acid production in the saliva. The completeness of the inhibition was dependent upon the concentrations used. In addition, the growth and acid production of a pure culture of *L. acidophilus* (Hadley) recovered from a carious lesion was adversely affected by dilutions as high as 1:4000 for indole and 1:2000 for indole-3-acetic acid. These and various clinical findings provided a working hypothesis whereby the relative absence of dental caries, especially of the smooth-surface type, in endemic pellagrins and persons with associated chronic nutritional deficiencies could, in part, be attributed to the products of protein putrefaction. Extensive periodontal disease is almost invariably seen in the mouths of these patients when the condition of nutritive failure is of long standing.[7] Smith and Moore[8] found that periodontal disease was apparent in every pellagrin examined by them and offered this factor as a possible cause of the disease. Spies[9] stated that practically all patients with severe pellagra of the oral cavity have "a secondary but extensive infection with Vincent's organisms."

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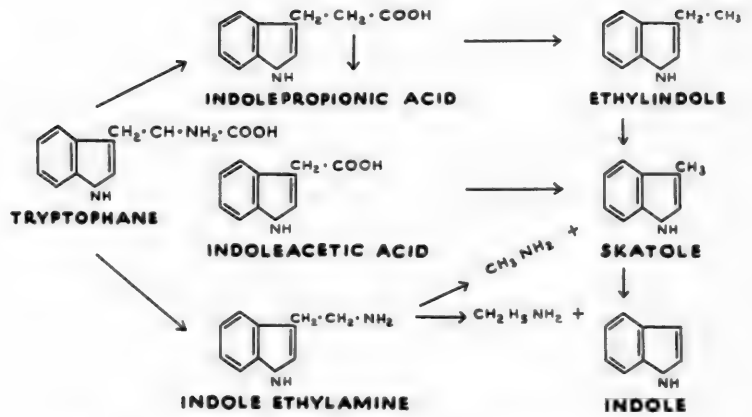

Fig. 1.—The products of bacterial action on tryptophane (Bodansky).

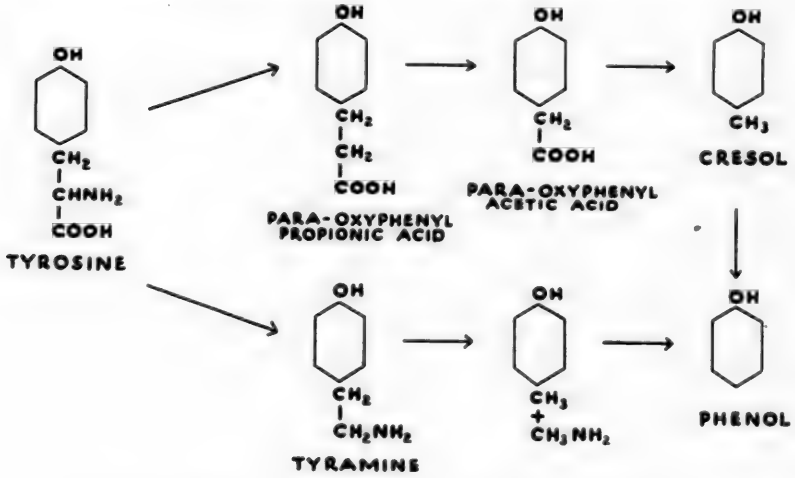

Fig. 2.—The products of bacterial action on tyrosine (Bodansky).

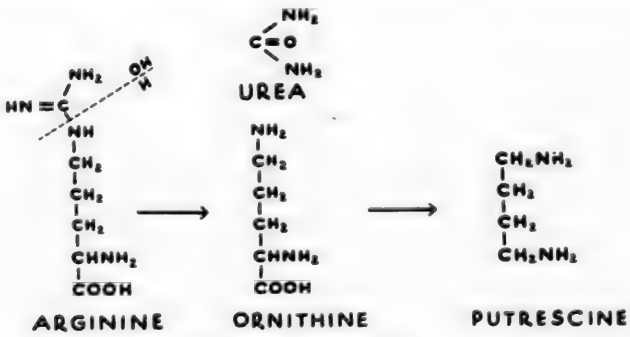

Fig. 3.—The products of bacterial action on arginine and ornithine (Bodansky).

In the present study, skatole, cresol, phenol, and a salt of putrescine (1,4-diaminobutane dihydrochloride) singly and in various combinations which included indole and indole-3-acetic acid were tested for (1) their properties as inhibitors of acid production in the saliva, and (2) their effect on the growth and acid production of a pure culture of the *L. acidophilus* (Hadley) recovered from a carious lesion. These compounds are all produced during the bacterial breakdown of proteins and have been reported as being present in the saliva of persons with periodontal disease.[10] (Figs. 1, 2, and 3.)

MATERIALS AND METHODS

The criteria for the selection of patients were as follows: (1) the presence of ten or more natural occluding teeth; (2) a history of dental caries susceptibility as determined from a clinical examination with a mouth mirror, exploring tine, and full-mouth or bite-wing radiograms; and (3) a recent presence of dental caries activity as indicated by the results of the Hadley,[11] Fosdick, Hansen, and Epple,[12] and Dreizen, Mann, Cline, and Spies[13] laboratory tests for dental caries activity.

The effect of the test compounds was determined on a total of 158 samples of paraffin-stimulated saliva submitted by seventy-three patients and twelve of the clinic personnel. The samples were collected in sterile flasks between 8 A.M. and 10 A.M. The chemical test for caries susceptibility of Fosdick, and associates[12] was chosen to demonstrate the action of the test compounds singly and in various combinations on acid production in the saliva. The method has been described in detail in an earlier publication.[6]

The effect of the test compounds, singly and in various combinations, on the growth and acid production of the oral lactobacilli was determined by the use of a pure culture of the *L. acidophilus* (Hadley) provided by the American Type Culture Collection (ATCC No. 4646). The manner of the preparation of the inoculum and of the yeast extract dextrose broth which served as the special medium has also been presented in full in the previous article.[6] Growth was determined by centrifuging the cultures in Hopkins vaccine tubes for ten minutes at high speed and then immediately measuring the packed cell volume. Acid production was determined by titration of the centrifugate with 0.1N NaOH to the pH of the control tubes for which a Beckman pH meter was employed. Each series was set up in triplicate and readings were taken at the end of twenty-four, forty-eight, and seventy-two hours.

OBSERVATIONS

The Action of the Test Compounds on Acid Production in Saliva

A compilation of the results obtained when skatole was added to the saliva samples in vitro is presented in Fig. 4. The amounts of skatole which were added ranged from 1.0 mg. to 7.5 mg. per 10 c.c. of saliva. Acid production was inhibited either completely or partially in thirty of the thirty-one samples in which dental caries activity was observed in the control aliquot. As expected, thirteen samples which were negative for acid production remained so following the addition of the skatole. Skatole was significantly effective as an inhibitor of acid production in amounts of 4.0 mg. and above.

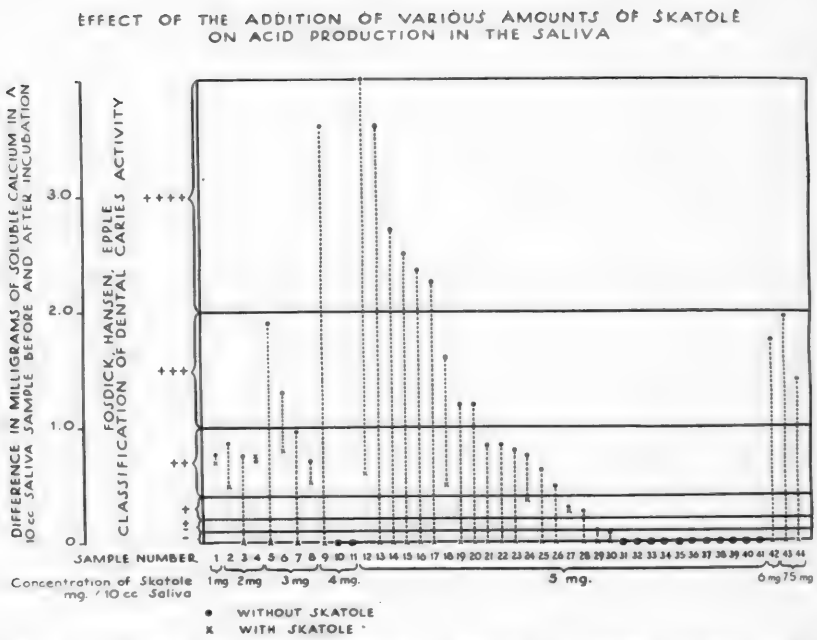
EFFECT OF THE ADDITION OF VARIOUS AMOUNTS OF SKATOLE ON ACID PRODUCTION IN THE SALIVA
DIFFERENCE IN MILLIGRAMS OF SOLUBLE CALCIUM IN A 10 cc SALIVA SAMPLE BEFORE AND AFTER INCUBATION
FOSDICK, HANSEN, EPPLE CLASSIFICATION OF DENTAL CARIES ACTIVITY
3.0
2.0
1.0
0
++++
+++
++
+
±
SAMPLE NUMBER
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
Concentration of Skatole mg. / 10 cc Saliva
1 mg
2 mg
3 mg
4 mg.
5 mg.
6 mg
7.5 mg
WITHOUT SKATOLE
WITH SKATOLE

Fig. 4.—The sample numbers are arranged in the order of decreasing activity in the control aliquot and not in the order in which the samples were collected. Skatole in the amounts used inhibited acid production in saliva partially or completely in thirty of the thirty-one samples in which dental caries activity was observed in the control aliquot.

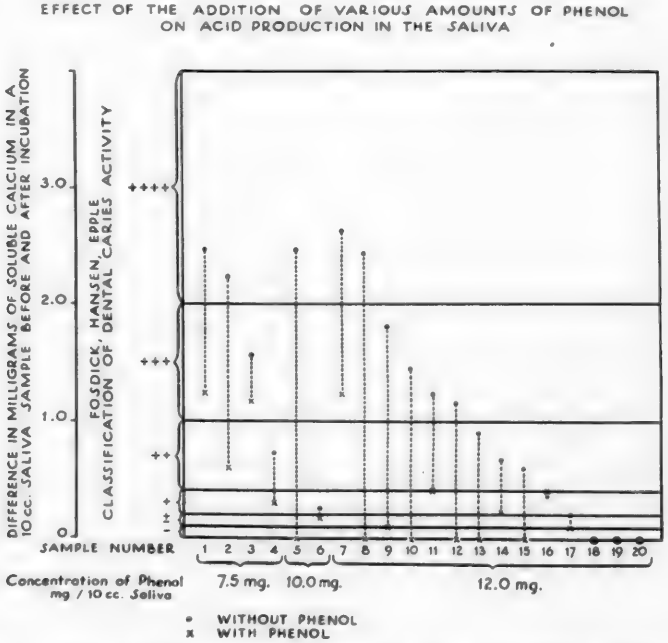
EFFECT OF THE ADDITION OF VARIOUS AMOUNTS OF PHENOL ON ACID PRODUCTION IN THE SALIVA
DIFFERENCE IN MILLIGRAMS OF SOLUBLE CALCIUM IN A 10 cc. SALIVA SAMPLE BEFORE AND AFTER INCUBATION
FOSDICK, HANSEN, EPPLE CLASSIFICATION OF DENTAL CARIES ACTIVITY
3.0
2.0
1.0
0
++++
+++
++
+
±
-
SAMPLE NUMBER
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Concentration of Phenol mg / 10 cc. Saliva
7.5 mg.
10.0 mg.
12.0 mg.
WITHOUT PHENOL
WITH PHENOL

Fig. 5.—The sample numbers are arranged in the order of decreasing activity in the control aliquot and not in the order in which the samples were collected. Phenol in the amounts used partially or completely inhibited acid production in sixteen of the seventeen samples in which the control aliquot was caries active.

Phenol was added to twenty samples of saliva in amounts ranging from 7.5 to 12.0 milligrams. The results are depicted in Fig. 5 where it will be noted that acid production was completely or partially inhibited in sixteen of the seventeen samples in which the control aliquot proved to be caries active. Three samples were negative and remained so following the addition of phenol. The most effective concentration of phenol of those used in this study as an inhibitor of acid production in saliva was 12.0 mg. per 10 c.c. of saliva.

Orthocresol and paracresol were added to twenty-eight and sixteen samples of saliva, respectively. Orthocresol was added in the amounts of 0.005 c.c. to three samples and 0.01 c.c. to twenty-five samples. Paracresol was added in 0.01 c.c. amounts to sixteen samples. In the amounts used, both orthocresol and paracresol inhibited acid production to some extent in all of the twenty and nine instances, respectively, in which caries activity was present in the control aliquot. The remaining samples were negative and remained so following the addition of cresol. These findings are shown in Fig. 6.

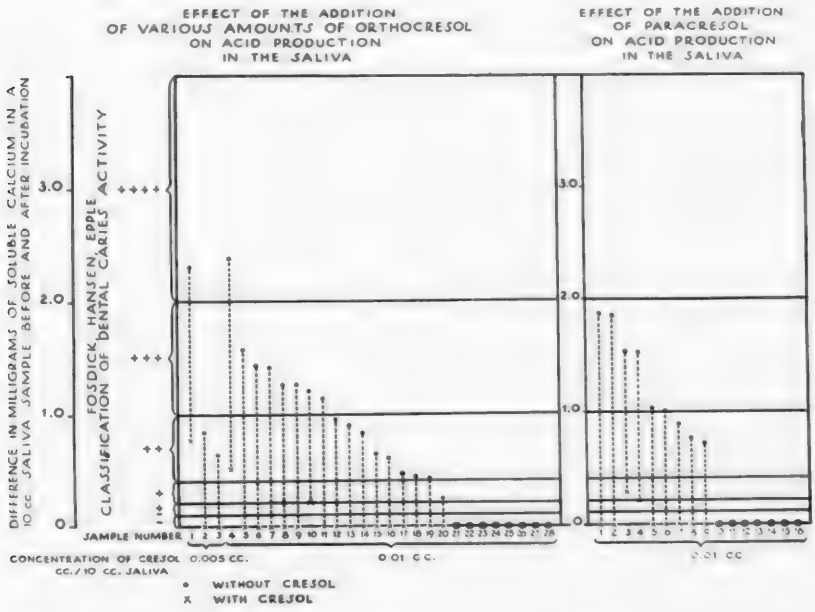

Fig. 6.—The sample numbers are arranged in the order of decreasing activity in the control aliquot and not in the order in which the samples were collected. Orthocresol and paracresol in the amounts used partially or completely inhibited acid production in all of the twenty-nine samples in which caries activity was present in the control aliquot.

Fig. 7 demonstrates the results obtained when the dihydrochloride salt of putrescine (1,4-diaminobutane dihydrochloride) was added to ten samples of saliva. There was total or partial inhibition of acid production in all of the samples in which the control aliquots were positive for caries activity. Four samples were negative and remained so following the addition of 1,4-diaminobutane dihydrochloride.

The results of adding various mixtures of the products of protein putrefaction to the saliva can be seen in Fig. 8. Mixtures were added to forty saliva samples. In all but one of the thirty-four samples in which the control aliquot showed caries activity, acid production was wholly or partially inhibited. Six samples were negative and remained so following the addition of the various mixtures. A very effective combination consisted of 2 mg. indole, 2 mg. indole-3-acetic acid, and 2 mg. skatole.

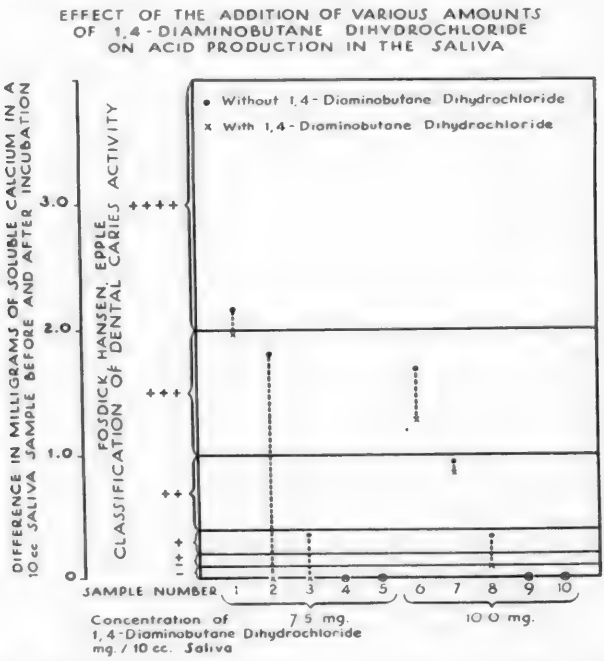
EFFECT OF THE ADDITION OF VARIOUS AMOUNTS OF 1,4-DIAMINOBUTANE DIHYDROCHLORIDE ON ACID PRODUCTION IN THE SALIVA
DIFFERENCE IN MILLIGRAMS OF SOLUBLE CALCIUM IN A 10 cc SALIVA SAMPLE BEFORE AND AFTER INCUBATION
FOSDICK, HANSEN, EPPLE CLASSIFICATION OF DENTAL CARIES ACTIVITY
• Without 1,4-Diaminobutane Dihydrochloride
x With 1,4-Diaminobutane Dihydrochloride
3.0
2.0
1.0
0
++++
+++
++
+
±
SAMPLE NUMBER 1 2 3 4 5 6 7 8 9 10
Concentration of 1,4-Diaminobutane Dihydrochloride mg. / 10 cc. Saliva
7.5 mg.
10.0 mg.

Fig. 7.—The sample numbers are arranged in the order of decreasing activity in the control aliquot and not in the order in which the samples were collected. 1,4-diaminobutane dihydrochloride in the amounts used partially or completely inhibited acid production in all six of the samples in which the control aliquot was positive for caries activity.

The Action of the Test Compounds on the Growth and Acid Production of the Lactobacillus Acidophilus (Hadley)

The results of the addition of the test compounds in various amounts, singly and in combination, on the growth and acid production of a pure culture of a strain of the *L. acidophilus* recovered from a carious lesion are presented in Tables I and II. The medium used was yeast extract dextrose broth, the incubation temperature 37° C., and the incubation periods twenty-four, forty-eight, and seventy-two hours.* It will be noted that skatole in amounts of 4.0 mg. and over completely inhibited the growth and acid production of the test organism throughout the experimental period. Amounts ranging from 1.0 to

*The first readings of the tubes containing phenol and 1,4-diaminobutane dihydrochloride were made at the end of thirty-six hours.

TABLE I

EFFECT OF SKATOLE, CRESOL (ORTHO- AND PARA-), 1,4-DIAMINOBUTANE DIHYDROCHLORIDE, AND PHENOL ON THE GROWTH AND ACID PRODUCTION OF THE LACTOBACILLUS ACIDOPHILUS (HADLEY)

TUBE	GROWTH (PACKED CELL VOLUME*)			ACID PRODUCTION (C.C. 0.1N NaOH		
	24 HOURS	48 HOURS	72 HOURS	24 HOURS	48 HOURS	72 HOURS
			Skatole			
Control	++	+++	+++	0.40	4.40	4.60
1.0 mg.	++	+++	+++	0.40	3.08	4.25
1.5 mg.	±	+++	+++	0.05	3.10	3.80
2.0 mg.	±	+++	+++	0.03	1.77	2.75
2.5 mg.	±	++	+++	0.10	1.10	2.75
3.0 mg.	–	–	+	0.00	0.00	0.60
4.0 mg.	–	–	–	0.00	0.00	0.00
5.0 mg.	–	–	–	0.00	0.00	0.00
7.5 mg.	–	–	–	0.00	0.00	0.00
			Orthocresol			
Control	+	+++	+++	0.40	3.85	4.45
1.05 mg.	±	+++	+++	0.02	3.00	3.55
2.10 mg.	–	++	+++	0.00	1.30	3.17
3.15 mg.	–	–	+	0.00	0.00	1.59
4.20 mg.	–	–	–	0.00	0.00	0.00
5.25 mg.	–	–	–	0.00	0.00	0.00
6.30 mg.	–	–	–	0.00	0.00	0.00
7.35 mg.	–	–	–	0.00	0.00	0.00
			Paracresol			
Control	+	+++	+++	0.58	4.18	4.40
1.04 mg.	+	+++	+++	0.30	4.10	4.10
2.08 mg.	±	+++	+++	0.01	3.13	3.00
3.12 mg.	–	++	++	0.00	1.08	2.55
4.16 mg.	–	±	++	0.00	1.00	1.75
5.20 mg.	–	±	++	0.00	0.50	1.50
6.24 mg.	–	±	+	0.00	0.45	0.80
7.28 mg.	–	–	±	0.00	0.10	0.30
			Phenol			
Control	++	+++	+++	3.00†	3.40	4.40
1.07 mg.	++	+++	+++	3.00	3.20	4.40
2.14 mg.	++	+++	+++	3.00	3.40	4.20
3.21 mg.	++	+++	+++	2.40	3.50	4.20
4.28 mg.	+	++	++	1.70	3.50	4.20
5.35 mg.	+	++	++	1.70	2.85	3.60
6.42 mg.	+	++	++	1.50	2.50	3.60
7.49 mg.	+	+	+	1.40	2.50	3.50
9.63 mg.	±	±	+	1.00	1.90	2.70
			1,4-Diaminobutane Dihydrochloride			
Control	+++	+++	+++	3.40†	5.20	5.20
1.0 mg.	+++	+++	+++	3.45	5.20	5.20
1.5 mg.	+++	+++	+++	3.45	5.30	5.20
2.0 mg.	+++	+++	+++	3.45	5.15	5.30
2.5 mg.	+++	+++	+++	3.00	5.50	5.30
3.0 mg.	+++	+++	+++	3.40	4.55	5.20
4.0 mg.	+++	+++	+++	3.40	4.80	5.30
5.0 mg.	+++	+++	+++	3.20	4.70	5.20
6.0 mg.	+++	+++	+++	3.50	4.80	5.30
7.5 mg.	+++	+++	+++	3.20	4.70	5.20
10.0 mg.	+++	+++	+++	3.20	4.70	5.10
15.0 mg.	+++	+++	+++	3.25	4.60	5.10

*0.0-0.1 c.c. –
0.1-0.25 c.c. ±
0.25-0.5 c.c. +
0.5-1.0 c.c. ++
1.0-2.0 c.c. +++
†36-hour reading.

TABLE II

EFFECT OF VARIOUS COMBINATIONS OF THE PRODUCTS OF PROTEIN PUTREFACTION ON THE GROWTH AND ACID PRODUCTION OF THE LACTOBACILLUS ACIDOPHILUS (HADLEY)

TUBE	GROWTH (PACKED CELL VOLUME*)			ACID PRODUCTION (C.C. 0.1N NaOH)		
	24 HOURS	48 HOURS	72 HOURS	24 HOURS	48 HOURS	72 HOURS
Control	+ +	+ + +	+ + +	1.00	4.15	4.70
Mixture No. 1 1.0 mg. indole 1.0 mg. skatole 1.0 mg. phenol 1.0 mg. para-cresol	±	+ + +	+ + +	0.10	2.35	3.00
Mixture No. 2 2.0 mg. indole 2.0 mg. indole-3-acetic acid 2.0 mg. skatole	–	–	–	0.00	0.00	0.00
Mixture No. 3 2.5 mg. indole 2.5 mg. indole-3-acetic acid 2.5 mg. phenol	±	+ +	+ +	0.20	1.20	1.50
Mixture No. 4 1.5 mg. indole 1.5 mg. indole-3-acetic acid 1.5 mg. skatole 1.5 mg. phenol	–	–	–	0.00	0.00	0.00
Mixture No. 5 1.0 mg. indole 1.0 mg. 1,4-diaminobutane dihydrochloride 1.0 mg. skatole 1.0 mg. indole-3-acetic acid	–	–	–	0.00	0.00	0.00

*0.0-0.1 c.c. –
0.1-0.25 c.c. ±
0.25-0.5 c.c. +
0.5-1.0 c.c. + +
1.0-2.0 c.c. + + +

3.0 mg. exerted a partial inhibitory effect, the degree being directly proportional to the amount of skatole added. Orthocresol was completely effective in amounts of 4.20 mg. and above. All smaller amounts exerted a partial inhibitory effect. The inhibitory power of paracresol was limited to amounts of 2.08 mg. and above. The effect in all instances was partial. Phenol was but slightly effective in quantities of 5.35 mg. and above. A significant partial inhibition occurred in the tubes containing 9.63 milligrams. The salt of putrescine, 1,4-diaminobutane dihydrochloride, when used in amounts ranging from 1.0 to 15.0 mg. was completely ineffective against this organism.

Five different mixtures were added to determine the action exerted when small quantities of these compounds were used in combination. Three exerted a complete inhibitory effect and two, a partial one. Mixtures which completely prevented the growth and acid production of the test organism were (a) 2.0 mg. indole, 2.0 mg. indole-3-acetic acid, and 2.0 mg. skatole; (b) 1.5 mg. indole, 1.5 mg. indole-3-acetic acid, 1.5 mg. skatole, and 1.5 mg. phenol; (c) 1.0 mg. indole, 1.0 mg. skatole, 1.0 mg. indole-3-acetic acid, and 1.0 mg. 1,4-diaminobutane dihydrochloride.

DISCUSSION

The results of these in vitro studies lend support to the working hypothesis that the products of the putrefaction of proteins may prevent the growth of the acidogenic and aciduric organisms associated with dental caries and thereby be responsible for a lowered incidence of dental decay. It is noteworthy that the action of these compounds appears to be additive since they were more effective as inhibitors of acid production in saliva when used in combination than when they were used alone in the same amounts by weight. For example, 1 mg. of skatole was not very effective when added alone, but when the same amount was added in combination with 1 mg. indole, 1 mg. phenol, and 1 mg. paracresol a significant decrease in acid production occurred (Fig. 8).

In periodontal disease the breakdown of the supporting tissues of the tooth occurs in the proximity of those areas on the tooth surface where smooth-surface caries is initiated. As a result of this process, indole, indole-3-acetic acid, skatole, phenol, cresol, putrescine, ammonia, and urea may be produced. Whether or not these compounds are elaborated in vivo in amounts sufficient to exert the effects demonstrated in these in vitro studies remains to be determined. It is conceivable, however, that in chronic periodontal disease much smaller concentrations may exert an inhibitory action since these compounds are produced over long periods of time near the sheltered areas of the teeth where the diluent action of saliva is not a very significant factor.

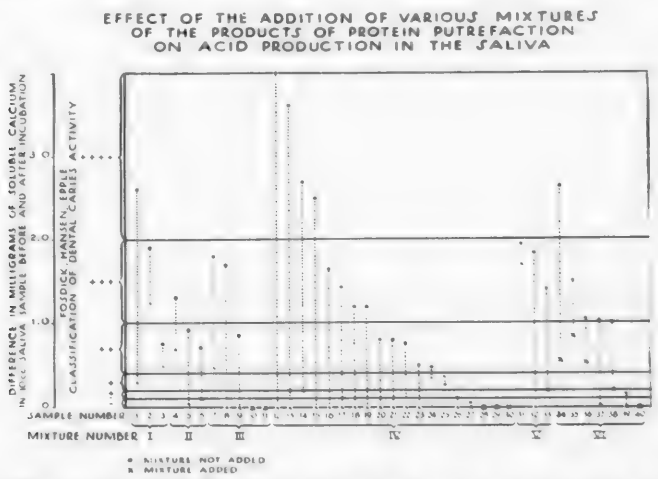
EFFECT OF THE ADDITION OF VARIOUS MIXTURES
OF THE PRODUCTS OF PROTEIN PUTREFACTION
ON ACID PRODUCTION IN THE SALIVA
DIFFERENCE IN MILLIGRAMS OF SOLUBLE CALCIUM
IN ROCK SALIVA BEFORE AND AFTER INCUBATION
FOSDICK HANSEN EPPLE
CLASSIFICATION OF DENTAL CARIES ACTIVITY
SAMPLE NUMBER
MIXTURE NUMBER I II III IV V VI
- MIXTURE NOT ADDED
+ MIXTURE ADDED

Fig. 8.—The sample numbers are arranged in the order of decreasing activity in the control aliquot and not in the order in which the samples were collected. The six mixtures of the products of protein putrefaction in the amounts used partially or completely inhibited acid production in thirty-three of the thirty-four samples in which the control aliquot was positive for caries activity.

Mixture Number

I. 1.0 mg. indole
1.0 mg. indole-3-acetic acid
1.0 mg. skatole
1.0 mg. phenol

II. 1.5 mg. indole
1.5 mg. indole-3-acetic acid
1.5 mg. skatole

III. 1.5 mg. indole
1.5 mg. indole-3-acetic acid
1.5 mg. skatole
1.5 mg. phenol

Mixture Number

IV. 2.0 mg. indole
2.0 mg. indole-3-acetic acid
2.0 mg. skatole

V. 2.5 mg. indole
2.5 mg. indole-3-acetic acid
2.5 mg. phenol

VI. 1.0 mg. indole
1.0 mg. skatole
1.0 mg. phenol
1.0 mg. paracresol

While these studies have been confined to the compounds elaborated during the bacterial decomposition of tryptophane (indole, skatole, indole-3-acetic acid), tyrosine (cresol, phenol), and ornithine (putrescine), it is of interest that other investigators have demonstrated that ammonia and urea have a deterring effect upon the process of dental caries. Jordan and Burrows[14] stated that ammonia is almost always liberated in amino acid breakdown. In 1935, Grove and Grove[15] reported that ammonia is an immunizing factor in dental caries, while more recently Kesel, and co-workers[16] found that the development of ammonia nitrogen in the oral cavity may be responsible for a decreased susceptibility to dental caries. Urea, in addition to being a constituent of the normal mixed saliva of human beings,[17] is produced in the conversion of arginine to ornithine by some proteolytic bacteria.[18] Stephan[19] has reported that urea may counteract the influence of carbohydrates on the pH of the dental plaque. When urea was employed as a dentifrice it resulted in a decrease in the incidence of new lesions and also retarded the progress of carious lesions already present.[20] In view of these findings and the results of the studies reported in this paper, it would appear that the products of protein putrefaction may play a significant role in lessening the susceptibility to dental caries.

SUMMARY AND CONCLUSIONS

1. Relatively dilute solutions of skatole, phenol, cresol, and a salt of putrescine (1,4-diaminobutane dihydrochloride) partially or completely inhibited acid production in vitro in eighty-one of the eighty-three saliva samples in which caries activity was found to be present in the control aliquot.

2. Dilute solutions of certain mixtures of these compounds, together with indole and indole-3-acetic acid, significantly inhibited acid production in vitro in all but one of the thirty-four saliva samples in which caries activity was present in the control aliquots. The action of these compounds appears to be additive, since they were more effective as inhibitors of acid production in saliva when used in combination than when used alone in the same amounts by weight.

3. When added in relatively small amounts, skatole, phenol, and cresol, singly and in various combinations with a dihydrochloride salt of putrescine, indole, and indole-3-acetic acid, significantly inhibited the growth and acid production of a pure culture of *L. acidophilus* (Hadley) recovered from a carious lesion.

4. These findings lend further support to the working hypothesis that the products of protein putrefaction may prevent the growth of the aciduric and acidogenic organisms associated with dental caries and thereby offer a partial explanation of the lowered incidence of dental decay associated with the high prevalence of periodontal disease in endemic pellagrins and persons with associated chronic nutritional deficiency diseases.

We are indebted to Miss Thelma Skinner for her aid in making the caries activity determinations.

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RESULTS OF A THREE-YEAR STUDY OF THE EFFECTIVENESS OF CARBOHYDRATE RESTRICTION IN REDUCING SALIVARY LACTOBACILLUS COUNTS

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ACTIVE dental caries has been found to be closely correlated with the consistent presence of large numbers of lactobacilli in the saliva; and conversely, lack of caries activity is correlated with few or no oral lactobacilli.[1-3] In cases of active dental caries, an effective reduction in the number of oral lactobacilli will be followed by an arrest of the caries activity. This reduction can be accomplished by the elimination of sugar and restriction of starches in the diets of caries-active individuals.[4-6]

On the basis of this knowledge, the University of Michigan School of Dentistry has for some years been operating a Caries Control Laboratory in which salivary lactobacillus counts are determined, caries activity diagnoses made, and low carbohydrate diets prescribed for caries-active cases. The technical procedure of making the counts and the diets recommended have been described by Jay.[7]

In October, 1944, the Ohio State University College of Dentistry opened a Caries Control Laboratory patterned closely after the Michigan laboratory. The technique of making the counts has been kept identical with that of Michigan, so that the results of the two laboratories should be comparable. The diets now used in the Ohio laboratory differ from the Michigan diets in that the fat content is lower at Ohio State. But both of the Plan I (basic) diets require a complete elimination of sugar and a limiting of other carbohydrates to approximately 100 Gm. daily. Such a diet excludes bread, potatoes, and certain fruits and vegetables of high carbohydrate content, as well as sugar, candy, pastries, and sweet drinks.

A patient with a high lactobacillus count (over 10,000 per cubic centimeter of saliva) is diagnosed as caries-active. Subsequent to repair of all carious areas, he is instructed to follow the Plan I diet for two weeks. Careful adherence to this low-carbohydrate regimen will nearly always lead to a drastic reduction in the number of oral lactobacilli. At the end of two weeks of the Plan I diet, a lactobacillus count is made and the patient is instructed to change to Plan II. This is merely the addition to Plan I of a limited amount of carbohydrates in the form of bread, potatoes, and an increased variety of vegetables and fruits. At the end of two weeks of Plan II, another salivary lactobacillus determination is made, and if the count remains low the patient is instructed to proceed to Plan III. This consists of Plan II with a further increase in carbohydrates in the form of greater variety of fruits and vegetables, and the equivalent of one teaspoonful of sugar at one meal a day if desired. The Michigan laboratory designates "sugar in desirable amount with one meal a day," but

For nine months of the operation of this laboratory the services of a technical assistant were financed by the Division of Dental Hygiene, Ohio State Department of Health.

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such instructions to patients have been found by us to lead, in some cases, to injudicious consumption of sugar with an accompanying rise in the number of oral lactobacilli. After a two-week period on the Plan III diet, the lactobacillus count is determined, and if the count remains low the patient is allowed to return to a nearly unrestricted diet, the only instructions being to keep sugar consumption very low (Plan IV).

DATA

Table I presents data made from October, 1944, to November, 1947, on the salivary lactobacillus counts of specimens from 559 individuals. With the exception of a small number of patients from the Dental College Clinic, these individuals are patients of private dentists scattered throughout Ohio and surrounding states. Laboratory reports on counts were sent to the dentists, and diets were prescribed through the dentists.

TABLE I

EFFECT OF DIETARY TREATMENT ON LACTOBACILLUS COUNTS OF PATIENTS TREATED THROUGH THE DENTAL CARIES LABORATORY OF OHIO STATE UNIVERSITY

		PATIENTS WITH REDUCED LACTOBACILLUS COUNTS		PATIENTS WITH INCREASED LACTOBACILLUS COUNTS		
	TOTAL NUMBER	NUMBER	PER CENT	NUMBER	PER CENT	REMARKS
Total number of individuals examined	559					
Low counts; dietary treatment not indicated	137					
High counts; dietary treatment indicated but not undertaken, or not yet started	206					
High counts; dietary treatment undertaken and Plan I successfully completed	216	216				
Completed Plan II	192	152	80	40	20	
Completed Plan III	138	95	69	43	31	
Completed 2 weeks on Plan IV	87	57	66	30	34	3 increased counts, later controlled
Checked after 3 months on Plan IV	48	29	60	19	40	2 increased counts, later controlled
Checked after 6 months on Plan IV	24	21	88	3	12	1 increased count, later controlled
Checked after 1 year on Plan IV	8	7	88	1	12	1 increased count, later controlled
Checked after 2 years on Plan IV	1	1	100	0	0	

Of the 559 examined, 137 were found to be low or negative for lactobacilli and were therefore diagnosed as caries-inactive. These individuals were not placed on a diet.

Of the total number examined, 422 were caries active as indicated by high lactobacillus counts (over 10,000 lactobacilli per cubic centimeter of saliva) and needed dietary treatment. Of this number, 206 are not included in Table I because (1) they are now in the process of Plan I, or (2) they failed to reduce the lactobacillus count by the Plan I diet and discontinued treatment, or (3) they did not wish to attempt dietary treatment for reasons ranging from lack of

interest or lack of willingness to cooperate, to inability to obtain required meat, eggs, and other high protein foods rendered unavailable by rationing, scarcity, and high prices.

Of the 422 needing the treatment, 216 have successfully completed the Plan I diet with satisfactory reduction in the number of oral lactobacilli. A few of the 216 patients dropped treatment after two weeks on Plan I. A number are still in the process of the following two weeks on Plan II and no further data are available for them at this time. But of the 192 who progressed from Plan I and have so far completed Plan II, 152 (80 per cent) maintained low counts at the end of two-week periods; forty (20 per cent) showed increases in lactobacilli. Of the 152 who successfully completed Plan II, 138 have completed Plan III. Of these, ninety-five (64 per cent) retained low lactobacillus counts at the end of two weeks. Forty-three (31 per cent) showed increased counts.

Of the ninety-five patients successfully finishing Plan III, eighty-seven have been on Plan IV (the diet unrestricted except for reducing sugar to a minimum) for at least two weeks. Of these, fifty-seven (66 per cent) maintained low counts; thirty (34 per cent) showed increases.

Forty-eight patients who successfully completed the entire dietary treatment have been examined at the end of three months on the Plan IV diet. Of these, twenty-nine (60 per cent) had maintained low lactobacillus counts; nineteen (40 per cent) had increased counts. Of twenty-four examined at the end of six months, twenty-one (88 per cent) had low lactobacillus counts; three (12 per cent) had increased counts. Of eight examined at the end of one year on the less restricted diet, seven had retained low counts; one had increased. The one patient checked at the end of two years on the less restricted diet following the course of treatment still retains a low lactobacillus count.

DISCUSSION

The figures given in Table I indicate the results of the Ohio State University Caries Control Laboratory in its dietary treatment for reducing the oral lactobacillus count as a measure in the control of dental caries. These results compare favorably with those given by Jay[6] for the Michigan laboratory. The percentages under the column "Patients with Reduced Lactobacillus Counts" are slightly lower than Jay's. The authors believe that this difference may be at least partially accounted for by the fact that during the entire time the Ohio laboratory has been in operation there has been rationing or scarcity, and very high prices of meat and other foods required on the low carbohydrate diet. In a number of unsuccessful cases investigated, it was found that it had been virtually impossible to adhere strictly to the diet. The numbers of individuals checked after three and six months, and one and two years on Plan IV will increase as time goes on and more patients are examined after longer intervals.

It should be noted that a number of individuals listed in the table as having increased counts after a certain length of time had subsequently controlled counts (the lactobacillus count reduced) by resuming dietary treatment.

One significant change has been made from the dietary instructions given by Jay. Jay[6] indicated that, following a course of dietary treatment, the patient

is "allowed to return to an entirely unrestricted diet." Individuals apparently differ in their ability to tolerate sugar without experiencing an increased lactobacillus count after dietary treatment. Investigation into the subsequent diets of individuals who have completed the course of treatment leads the authors to believe that those who voluntarily maintain a low-sugar diet will retain a low lactobacillus count over a longer period of time than those who promptly resume a high-sugar diet. Therefore, rather than allow the patient to return to an "entirely unrestricted" diet, it is recommended that the sugar intake be held to a very small amount.

Our figures for patients whose lactobacillus counts were checked after one and two years are too small to give the percentages any significance.

SUMMARY

Of specimens from 559 individuals examined for active dental caries as indicated by counts of oral lactobacilli, 137 individuals were found to be caries inactive, and 422 caries active. Of the caries-active, 216 successfully completed Plan I of the dietary treatment. Of these, 138 have so far completed the course of treatment ninety-five of these finishing with low lactobacillus counts. Twenty-four of the ninety-five had been examined six months later, and twenty-one had retained low counts. Eight of these twenty-one had been examined after one year following treatment, and seven of these had retained low counts. One individual was examined after two years following dietary treatment and the count was low. These results compare favorably with those recently presented by Jay.

The authors wish to express appreciation to Betty Miller Forsythe for technical assistance, and to Dr. H. B. G. Robinson for numerous consultations.

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A STUDY OF THE PHOSPHATE SOLUBILITY OF THE HUMAN ENAMEL SURFACE

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THE solubility of the enamel has been the subject of a considerable number of investigations. These have been extensively reviewed in an earlier publication.[1] Previous measurements of the solubility of the enamel surface have generally made use of methods which involve decalcification of a considerable thickness of the enamel surface. Head[2] approached this problem by devising a machine which would measure within 0.1 micron how far a given force may drive a standard plunger into sound and partly decalcified enamel. However, intact enamel surfaces could not be tested on this machine since readings could be taken only on surfaces that had been ground to a plane. Later, Pickerill[3] and Dobbs[4] employed sharp instruments and judged roughly the softening or decalcification from the amount of enamel which could be scratched off. More recently, Hoff and Køszeg[5] investigated the solubility of certain areas of the enamel surface with an apparatus similar to that of Head. However, the technic they utilized was accurate only to within 3 to 4 microns.

These methods, and gravimetric procedures used by Pickerill[6] and Thurlow and Bunzell,[7] have insufficient sensitivity to be applicable to the problem which we wished to investigate. Our purpose was to study the factors affecting the superficial solubility of a portion of the enamel surface of a single tooth.

Phosphorus is one of the principal components of the enamel, comprising 17 to 18 per cent of the inorganic matter. Even a slight decalcification of the enamel surface should cause phosphate to go into solution. Our method for measuring surface solubility is based on one of the very sensitive methods available for the determination of phosphate. Whether the ratio of calcium and phosphorus in solution will be the same as that in enamel cannot be answered at present. In decalcification of powdered enamel Benedict and Kanthak[8] observed that the Ca/P ratio in solution at first was higher than in the enamel. Later it became identical with enamel. Undoubtedly, a more complete evaluation of the decalcification process could be attained with a combined determination of Ca and P than of P alone. Our data are, therefore, presented with the hope that a more comprehensive chemical study may be added later.

METHOD

In order to assure a standard area for the test, a disc of tin foil, 4 mm. in diameter and 0.070 mm. thick, was made with a puncher and adapted to the surface of the tooth to be tested. While the tooth was observed under a microscope, blue inlay wax was melted to the edges of the foil, and the entire tooth was covered with wax. The tin foil was quickly removed with a piece of heated gutta-percha attached to a hand instrument. Windows prepared by this tech-

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nic could be made on reasonably curved surfaces as well as on flat surfaces. Whenever more than one area was tested on the same tooth, the other areas were protected with tin foil before the crown was covered with wax in order to prevent contamination of the surface to be used later. A wax base was added to the tooth in order to keep the window facing upward during the period of decalcification.

The waxed tooth was placed in a cylindrical tube, 2.5 by 5.5 cm., which was half submerged in a constant temperature water bath at 37° Centrigrade. In this vessel the tooth was subjected to 5 c.c. of 0.2 molar acetate buffer at pH 4 for ten minutes with constant agitation by a mechanical stirrer. After exposure to the buffer, the tooth was removed and two aliquots of 1.5 c.c. of buffer were withdrawn for P determination. To each of the aliquots were added 2 c.c. of 5 per cent trichloracetic acid, 0.3 c.c. of ammonium molybdate, and 0.3 c.c. aminonaphtholsulfonic acid made up according to Hoffman's modification of Fiske and Subbarow's method.[9] The solutions were mixed, kept in darkness for ten minutes, and then their light absorption determined with a Ceneo photelometer, a red filter being used. The values were read from a calibration curve which was drawn daily from a blank and on two known amounts of phosphorus. This method was capable of detecting the phosphate content of samples containing six micrograms to within 0.5 per cent, the average deviation of ten samples being 1.2 per cent.

The teeth used in this study were scrubbed with a clean toothbrush after extraction and stored in a humidor. Although storing had no apparent effect on the solubility, we attempted to finish our tests within a week. All unerupted teeth included in the study were fully formed.

Four phases of the surface solubility problem were studied. Initially, we investigated the changes in solubility of a single area during repeated series of exposures. Likewise, the variation in solubility of different surfaces on the same teeth were studied along with a comparison of solubilities of surfaces of different teeth. Finally, we have compared the solubility of natural enamel surfaces and those that have been specially ground.

RESULTS

A single solubility test removed on the average about 7 micrograms of phosphorus. This would correspond to an enamel thickness of approximately 1 micron, depending upon the amount of calcium which is also dissolved. Hence, the repeated tests on intact enamel surface are concerned with an extremely superficial structure.

The left side of Fig. 1 compares four consecutive runs on thirty areas in twenty-three permanent teeth. The teeth included in this series were of all types and from patients of both sexes, ranging in age from 18 to 69 years. The average P values in gamma for the four runs were 7.3, 9.4, 9.9, and 10.2. The increasing values of the averages express a trend which was consistent for the majority of the teeth. In 80 per cent of the teeth the first run had a lower value than the second run, and in 85 per cent the first run had a lower value than the third and the fourth runs. The significance of the difference between the first and second run was 35 to 1, between first and third it was 195 to 1, and between

the first and fourth, 516 to 1. It was calculated by dividing the difference of the means by the standard error of the difference of the means. There was usually no great numerical difference in the level of the four runs. Thus, if the first run had a low value, as a rule the other runs also had low values, indicating that the phosphate solubility of that area was low for some distance inward.

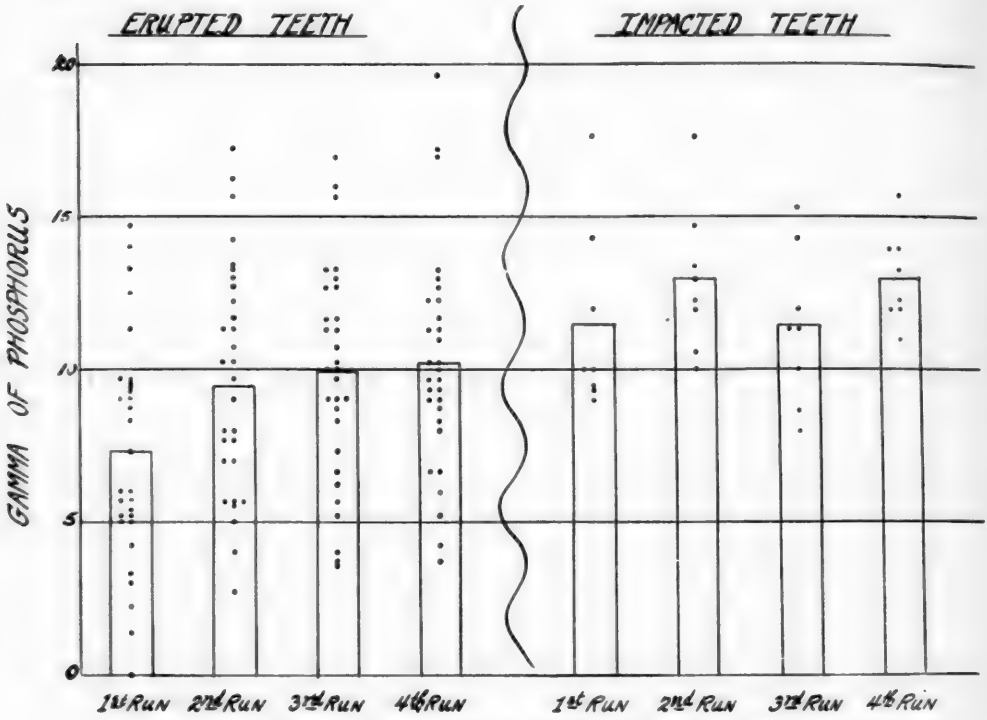

Fig. 1.—Four consecutive ten-minute runs on the same area in acetate buffer pH 4.0.

A few unerupted teeth were also studied. The results on eight areas in four unerupted teeth are plotted on the right side of Fig. 1. Here the increase in phosphate solubility from the first to the fourth run is not so striking. The phosphate solubility increased from the first to the second run in 75 per cent of the surfaces, but the significance of the difference was only 3 to 1. There was no difference between the averages of the first and third run, and the significance of the difference between the first and fourth run was only 4.5 to 1.

Solubility of Different Areas in the Same Tooth:

In decalcification of teeth for histological purposes it has frequently been observed that the enamel surface of erupted teeth dissolves unevenly. Superficial lesions of arrested caries have been found relatively resistant to acids,[10] and abraded surfaces are more soluble than intact enamel surfaces.[4] Fig. 2 shows that intact surfaces on the same tooth may also vary in solubility. The maximum percentage difference in solubility is plotted for each of thirteen

erupted and seven unerupted teeth. The plotted figures were arrived at by the testing of two or more areas on the same tooth and computing of the maximum percentage deviation from the average P solubility of these areas.

In all cases the solubility varied from one area to another, but the variation was small in the majority of the erupted teeth. The unerupted teeth tended to be more uneven in surface solubility, as expressed by the average deviation in P solubility which was 2.8 gamma (22.8 per cent) for the unerupted teeth, and 1.5 gamma (16.7 per cent) for the erupted teeth.

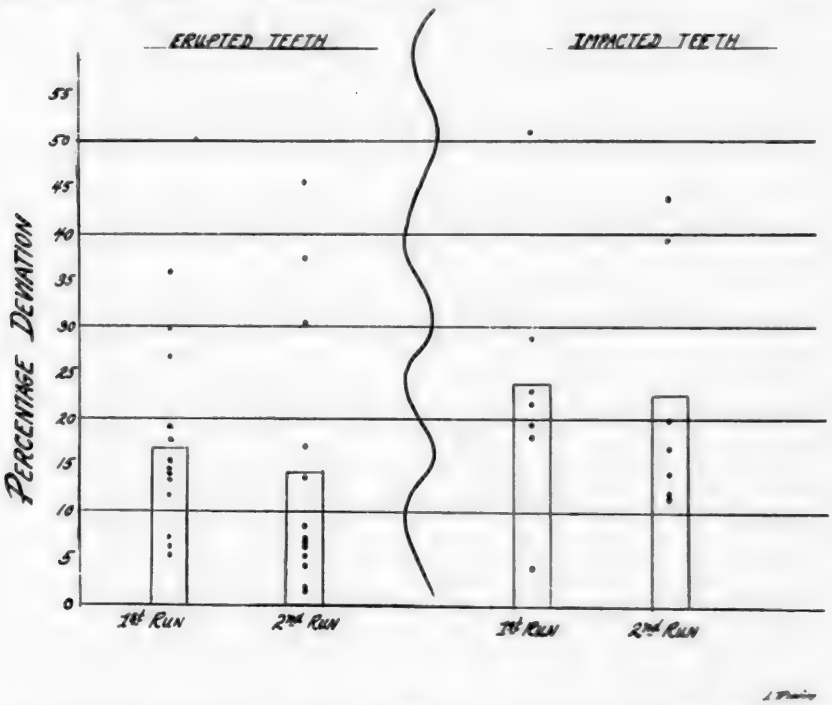

Fig. 2.—Percentage difference in solubility of different areas on the same tooth. Two ten-minute runs in acetate buffer pH 4.0.

All areas included in this series were given two exposures to the buffer. In the second exposure there was also a difference in the solubility from one area to another, but there was no relation between this difference and the difference found between the same areas in the first exposure. This further suggests incongruence in the solubility of the enamel surface. In four teeth, tests were repeated four times on different areas. The variation in surface solubility was apparent throughout the four runs, showing again that the variation is not strictly superficial. Buccal, lingual, and proximal surfaces were included in this study. Our data do not indicate any correlation between surface solubility and location of tooth surfaces.

Surface Solubility of Different Teeth:

The variation in surface solubility among different teeth was often found to be greater than that of different areas of the same tooth. Records were kept

of the age and sex of the individuals, the type of the tooth, surface roughness, pigmentation, caries, and fillings. Except for age and pigmentation, no correlation was found between those factors and surface solubility. Normal surfaces on carious teeth did not differ from surfaces of noncarious teeth in solubility. Low solubility was often associated with a dark brown stain, but the correlation was not conclusive.

In Fig. 3 there are comparisons of the P solubility among deciduous teeth, unerupted permanent teeth, and erupted permanent teeth from persons of different age groups. Two tests were made on thirteen deciduous, eleven unerupted, and fifty erupted teeth. The second run on a surface averaged higher than the first run for all groups, and the variation was about the same for each group as the standard deviation ranged merely from 3.5 to 3.9.

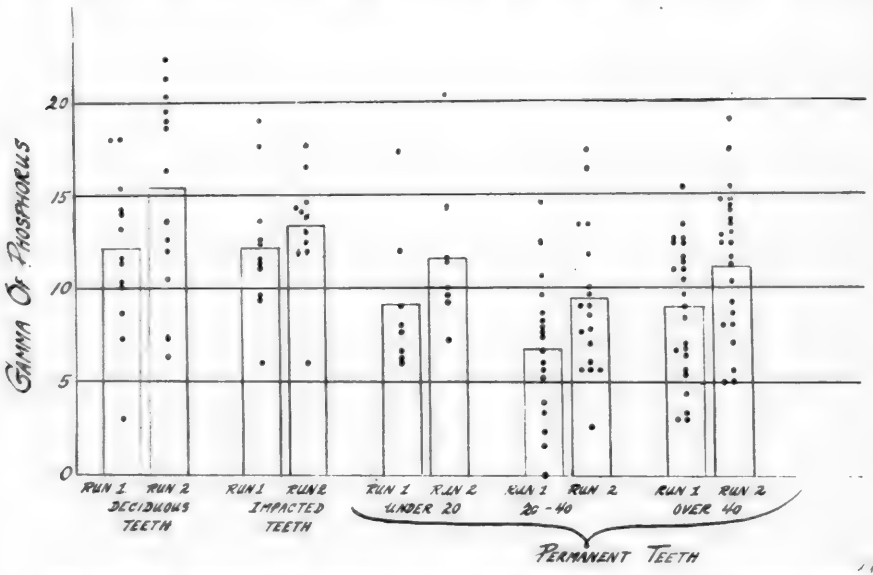

Fig. 3.—Two consecutive ten-minute runs in acetate buffer pH 4.0 on the same window.

Erupted deciduous enamel has a solution rate rather similar to unerupted permanent enamel. Erupted permanent teeth are about two-thirds as soluble as the aforementioned groups, indicating that there is a posteruptive decrease in P solubility. This decrease may be gradual, since teeth from persons 20 to 40 years of age were less soluble than those under twenty. Teeth from the oldest group, which averaged 56 years of age, appeared to have a solubility similar to the youngest group of erupted teeth. The mathematical significance of these differences has not been tested, but it seems unlikely that the differences between deciduous and permanent teeth and between erupted and unerupted teeth are due entirely to chance.

Solubility of Intact and Ground Enamel Surface:

Since there is a variation in the surface solubility of different areas on the same tooth, a comparison of the solubility of the intact and ground enamel

surface must be made in the same area, involving as far as possible the same enamel prisms. Fig. 4 shows a device which we have used in reproducing the window position on ground surfaces.

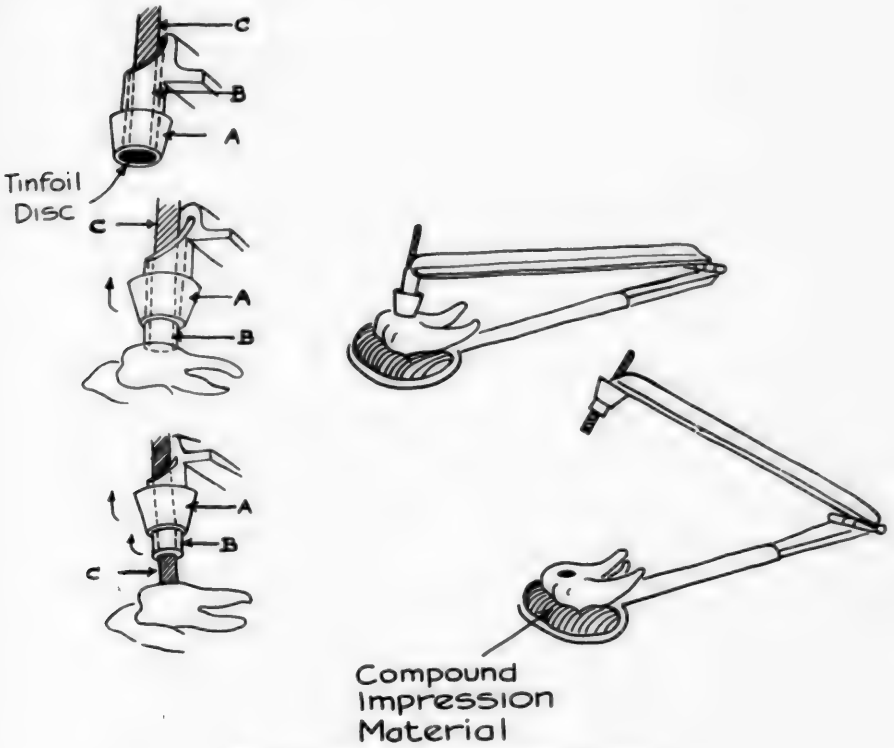

Fig. 4.—Device to reproduce window position.

The tooth is placed in soft compound in the desired position and in such a way that it may be replaced in the same position at will. The tin foil is placed in the movable cylinder, *A*, which has the same diameter as the tin foil and which slides up and down on a second cylinder, *B*, which is connected with the tooth through a hinge. The cylinder *A* with the foil is brought in place on the tooth surface and the foil is retained through digital pressure on rod *C*. Cylinders *A* and *B* may now be lifted, the foil adapted to the tooth surface and wax melted to the edges, and the technic described earlier followed through.

The surface was ground with a sandpaper disk and polished with a wet, felt wheel pumice and tin oxide until the surface was shiny and showed no evidence of scratches under the binocular microscope.

Fig. 5 shows the results of two consecutive runs on intact and ground surfaces from ten erupted teeth (24 to 66 years of age) and five unerupted teeth. The P solubility of the ground surface was considerably greater than the solubility of the intact surface in all cases except one. In six of the erupted teeth the same area was ground twice, and in five of these the P solubility of the second ground surface was greater than the first suggesting that the solubility

of the enamel increases toward the dentino-enamel junction. We did not control the thickness of enamel which was ground away and were therefore unable to evaluate the difference in solubility between natural and ground surfaces in different teeth. But the averages 10.2 and 20.6 gamma for the erupted and 11.1 and 24.0 for the unerupted teeth show that the difference is pronounced in both groups. In either case the significance of the difference was calculated to be more than a million to one.

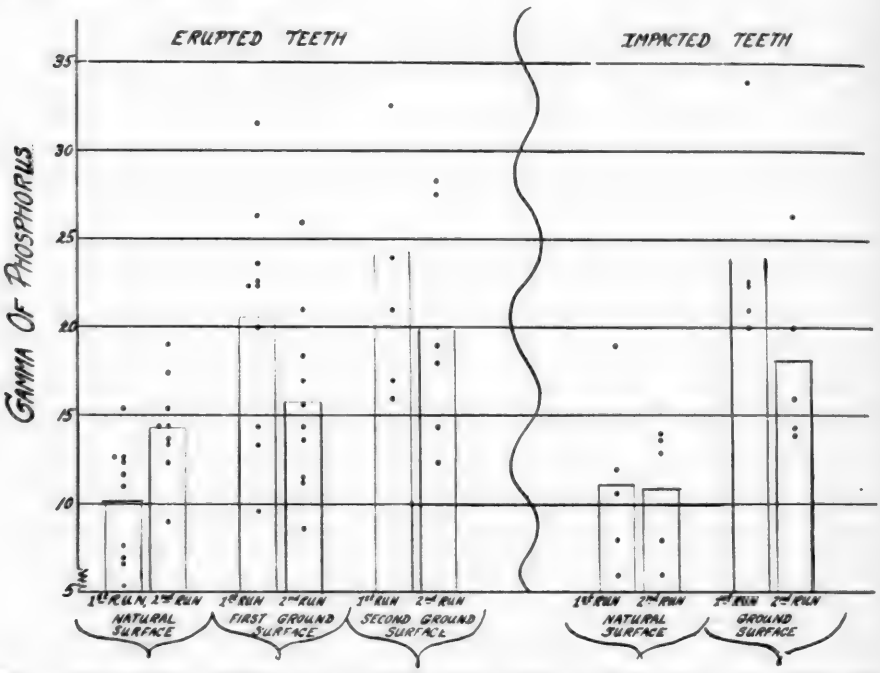

Fig. 5.—Comparative solubility of the natural enamel surface and the same area ground and polished. Two consecutive ten-minute runs in acetate buffer pH 4.0.

There was a considerable variation in the solubility of the ground surfaces in different teeth. This difference tended to be more marked in the erupted teeth. There was no correlation between the surface solubility and the solubility of the subsurface enamel.

Another consistent difference between intact and ground surfaces was disclosed by the two repeated exposures to the buffer. Whereas the first exposure gave a lower P solubility than the second in the majority of the intact surfaces, the first exposure of the ground surfaces gave a higher P solubility than the second in nineteen of twenty-one cases.

DISCUSSION

It should be emphasized that the rate of appearance of phosphate in acid may not always be a measure of the solution rate of the enamel from which the phosphate is derived. In addition to phosphate, the enamel contains calcium, carbonate, and magnesium in appreciable percentages.[11] These substances may be leached out by the buffer at rates which differ from that of the phosphate.

For example, hydroxyapatite might be converted to mono- or dicalcium phosphate by the equation:

$$3Ca_3(PO_4)_2 \cdot Ca(OH)_2 \xrightarrow{8HAc} 6CaHPO_4 + 4CaAc_2 \cdot 2H_2O$$

Then the calcium phosphorus ratio of the solution will depend upon the rate at which these two substances dissolve and diffuse into the acid. If calcium acetate alone goes into solution, no phosphate could be found. The same would be true in case carbonate salts dissolve. If calcium acetate and calcium phosphates dissolve at proper rates, calcium and phosphate ions would appear in solution in the same ratio as they are found in enamel; under these circumstances the phosphate method would be a good measure of the solution rate of enamel. If dicalcium phosphate is produced during a first test and is not all dissolved, it would accumulate and increase the amount of phosphate which appeared during the second test.

This hypothesis may explain the finding that acid generally dissolves more phosphate during the second test on a surface than it did during the first test. It might also be true that the outermost layer of the enamel is actually less soluble than the layer immediately underneath, but the final answer to this question requires a knowledge of the solution rate of calcium from enamel. In order to investigate this question a micromethod for calcium is being developed and some information on the calcium-to-phosphorus ratio of solutions of surface enamel may be presented later.

Our data tend to support the hypothesis that the difference observed between the phosphate solution rate of erupted and unerupted teeth is attributable to organic deposits on the teeth. Probably it is not related to the enamel cuticle, for Chase found that this structure was lost soon after eruption.[12] In its stead, there appeared an organic structure which he believed was of bacterial origin. This appeared in 84 per cent of the teeth which he examined. It may be coincidental that 80 per cent of the erupted teeth which I studied showed a lower solubility during the first than during the second test, or it may be an indication of a transient organic membrane on the surface of the 80 per cent showing this phenomenon.

Protein plaques are found on erupted teeth and the lowered solubility of the intact surfaces has been considered due to their presence by other workers. Dobbs[13] observed that ground surfaces of enamel were softened more rapidly by acids than intact surfaces. He assumed that the protective coating on intact enamel was a protein because it could be removed by digestion with trypsin. He observed microscopically that decalcification occurred first on the crests of the imbrication lines, and assumed that the protein in the grooves prevented decalcification there. If protein were absent from the surface, cracks in the enamel and surface roughness of the enamel would be expected to produce a higher surface solubility. Since no such increase was found, it seems likely that protein deposits were concerned with the lowered solubility of intact enamel surfaces.

A posteruptive reduction in the solubility of enamel was noted by Pickerill[8] and Dobbs.[4] The data presented here substantiate the posteruptive drop in

solubility but also suggest that further gradual change may take place. Teeth from persons between 20 and 40 years of age averaged 25 per cent less in surface solubility than teeth from persons under 20 years of age. This reduction may be due to changes either in the amount or nature of organic deposit or in the nature of the inorganic salt. Solubility may change because of reaction with fluoride[14] or other substances which have been shown to reduce the solubility of powdered enamel.[15]

Pickerill[3] believed there was an inverse relation between the density and hardness of the enamel and the rate of decalcification. He divided teeth on the basis of their appearance into "sclerotic" and "malacotic" types, and found that "sclerotic" enamel was denser, harder, but less soluble in acid than "malacotic" enamel. The present results do not support this finding, for no relation was found between the appearance of the teeth and the surface solubility. However, in his tests, Pickerill exposed the teeth to acids for periods ranging from twenty-four hours to three days, after which time a considerable thickness of the enamel must have dissolved. His results are therefore not comparable to the data presented in this paper, which involve the superficial structure of the enamel.

This same difficulty prevents the making of a comparison between the acid solubility of the immediate surface of enamel and its hardness and density. The only data available on these properties in the literature were obtained on subsurface enamel. The present data on the subsurface enamel revealed that the P solubility increased toward the dentino-enamel junction. This is in agreement with Dobbs'[4] finding that acid resistance decreases in the same direction. Karlstrøm[16] and Hodge[17] found that the hardness was greatest for the enamel near the external surface and decreased progressively toward the dentino-enamel junction and Karlstrøm found a similar decrease in density. When these results are compared with the present findings it appears that hardness and density are inversely related to acid solubility. It is possible that inner enamel is slightly more porous or permeable than external enamel, and the greater porosity facilitates penetration of acid and discloses itself by lowering the hardness and density.

It was unexpected to discover that the solubility of surface enamel bore no relation to the solubility of the enamel underneath. This lack of correlation suggests that the solubility of the surface depends not upon the original structure of the enamel as it was laid down, but upon protein deposits or chemical changes which were acquired after eruption of the teeth.

There is an important difference between the results of our findings on intact enamel surfaces and those by other workers on the solubility of powdered enamel. The work on intact surfaces shows an increase in solubility with consecutive runs. Repeated runs on powdered enamel showed decreasing solubility.[8, 18] This same phenomenon was observed for the ground surfaces we tested. Apparently, this phenomenon is a characteristic of a newly ground or prepared surface of enamel. Several agents have been tested as to their ability to reduce the solubility of powdered enamel.[19] In view of the difference between the solubility properties of the surface and the subsurface enamel, it would be of interest to learn whether or not these agents also affect the solubility of the intact enamel surface. For example, lead fluoride was found to be more effective than

sodium fluoride in reducing the solubility of powdered enamel, but when it was applied topically it was found to be ineffective in reducing dental caries.[20] Perhaps this discrepancy may be attributed to the inability of the lead ion to react with the intact enamel surface in the same manner that it reacts with powdered enamel particles.

SUMMARY

A quantitative method for the estimation of the phosphate solubility of intact enamel surface has been developed. Windows 4 mm. in diameter were exposed to the action of 5 c.c. of a sodium acetate buffer, pH 4, for the period of ten minutes each. The buffer, containing on the average 7 to 8 micrograms of phosphate, was analyzed by the Hoffman modification of the Fiske-Subbarow method.

Variations in phosphate solubility were found from area to area on the same tooth and between various areas on different teeth. On the average, deciduous and unerupted permanent teeth were more soluble than erupted permanent teeth. Ground surfaces were more soluble than intact enamel surfaces in all cases. The first test on an intact surface averaged lower in solubility than the second test. The reverse was true for ground surfaces.

The author wishes to acknowledge his indebtedness to Dr. Richard S. Manly for numerous helpful suggestions, and to thank Miss Eleanor Jeffrey for carrying out many of the phosphate determinations and Mr. Erling Johansen for making many of the wax windows.

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INTRODUCTORY OBSERVATIONS OF THE PERIODONTIUM OF THE SYRIAN HAMSTER

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PERIODONTAL disease has been observed in young hamsters raised on fine caries-producing diets and in older stock animals.[1, 2] Since hamsters appear to be highly susceptible to periodontal disturbances under certain laboratory conditions, they may prove to be useful experimental animals for the study of this disease.

This report is concerned with gross and histological observations of the apparently normal hamster periodontium, and gross and microscopic findings in animals with obvious periodontal disease.

METHODS

In order to study the normal periodontium of the hamster, twenty-five animals of the same birth date and of near sex and litter-mate distribution were raised under stock conditions on laboratory chow with weekly supplements and on bedding of wood shavings. They were sacrificed five at a time at approximately monthly intervals (with the exception of one two-month interval) over a five-month period. Age at death ranged from two to seven months. Celloidin sections[3] were prepared of the molar teeth in mesiodistal, buccolingual, and occlusal planes. In addition, the jaws of eleven random animals of unkown history were studied histologically, and gross observations were made of the jaws of many animals of all ages.

OBSERVATIONS

Grossly, the gingivae of the normal young living adult appears as a ledge of pink adherent tissue around the teeth, at a level about one-third of the height of the crown above the cemento-enamel junction. It can be stripped with ease with the aid of a fine explorer. Under magnifications up to 30 times there is no discernible departure from the general appearance of human gingivae.

Stock animals at about twenty-five days of age exhibit varying degrees of accumulation of debris and inflammation around the first and second molars and in the (unerupted) third molar region. By about the thirty-fifth day, the third molar has completely erupted and gross evidence of inflammation pres-

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ent during eruption, has disappeared. In older animals, above one year, marked attrition is seen with accompanying gingival recession and not infrequent exposure of the bifurcations of the multirooted teeth.

A severe type of periodontal disease (Fig. 1) is found among a very high percentage of the older animals of the stock colony. The brown material which is packed into the deep periodontal pockets and alveoli of missing teeth appears to consist chiefly of cellulose fibers and hair. Old age seems to be one of the most constant coexistent factors in this form of the disease. Dental caries is rarely found in hamsters maintained on this dietary regimen.

Fig. 2 is representative of the buccolingual sections of the maxilla in an animal eight weeks of age. The height of the gingivae and the absence of inflammation are evidence of the healthy, normal condition. The wide pulp canals are indicative of the immaturity of the teeth.

Fig. 1.

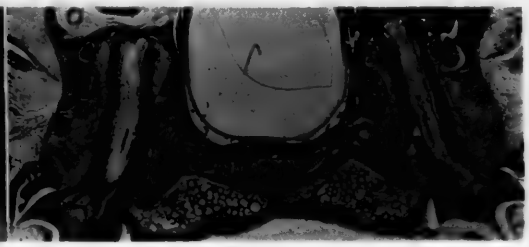


Fig. 2.

Fig. 1.—Maxilla of old stock animal. Destruction of palatal gingivae, with exposure of roots and buccal displacement of upper left third molar, is evident. Pocket around upper right second molar is filled with debris.

Fig. 2.—Buccolingual section through maxillary second molars of a young female (eight weeks). Note height of gingivae, wide pulp canals, and apical foramina. (×22 orig. magnification.)

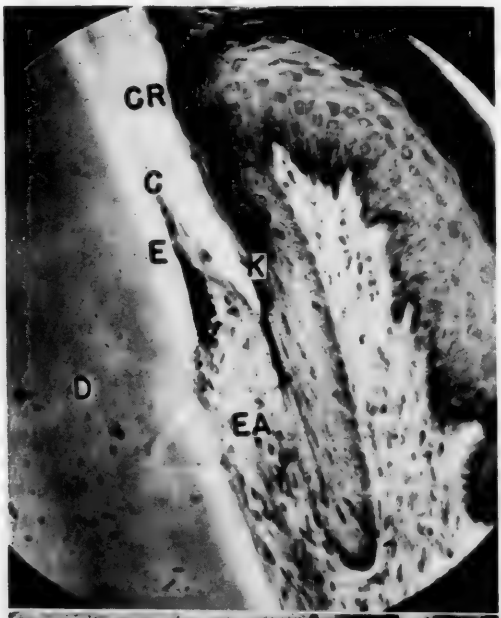
The epithelial attachment (in the absence of local pathosis) on the buccal and lingual surfaces of all the molars, and on the mesial of the first, and the distal of the third is represented by Fig. 3. To date it has not been determined conclusively whether or not the space between the secondary cuticle and the keratin separatory layer represents the gingival crevice. This "crevice" may be the "intraepithelial fissure" of Weski[4] and Euler,[5] an artifact of histologic preparation as believed by Orban,[6] or the physiologic crevice as considered by Beeks[7] in studies of man and animals other than the hamster. The condition pictured was found in most of the sections taken from the normal gingivae of the "non-contactual" surfaces of the molars. Fig. 4 is offered as illustration of this fact. It represents the findings in horizontal sections cut parallel to the occlusal plane. Multiple sections in this and other planes indicate that the keratin separatory layer does not extend completely through the interproximal region of the gingivae. In general, these findings are similar to those observations made of the gingival tissues of the rat.[8]

In a mesiodistal section of the mandibular molars of a 12-week-old female (Fig. 5), a normal and an abnormal interproximal area may be seen. Note the near vertical axes of the teeth in the plane of this section. The periodontal

membrane, alveolar bone, and teeth are reasonably comparable to the same structures in the human being.

A magnification of the diseased area shown in Fig. 5 is illustrated in Fig. 6. Accumulations of cellulose, hair, and epithelial and inflammatory cells fill the interproximal area. Inflammation is demonstrated in the underlying tissues, and resorption of the alveolar crest of bone is indicated by the presence of osteoclasts. This early form of disease was rare in the animals under seven months of age.

Fig. 3.



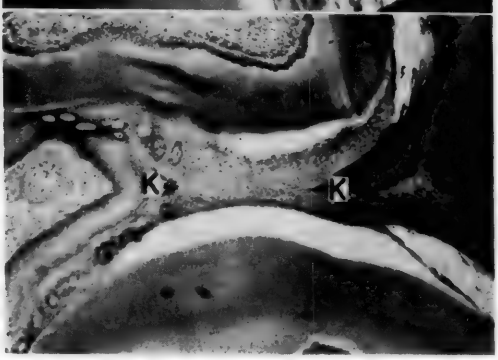


Fig. 4.

Fig. 3.—Epithelial attachment to lingual surface of upper molar of eight-week-old hamster. *D*, Dentin; *E*, enamel space; *C*, secondary cuticle; *EA*, epithelial attachment; *CR*, gingival crevice (?); *K*, keratin layer continuous with that of the oral epithelium; and prehyaline granules. (×430 orig. magnification.)

Fig. 4.—Interproximal area. Horizontal section in cervical region of two maxillary molars. Keratohyaline layer (*K*) extends toward but not across interproximal gingivae. (×100 orig. magnification.)

To check the impression that the method of housing in the presence of a soft diet seemed to influence the inception and/or course of periodontal disease,[1] several surveys were made. When hamsters have been sacrificed in this laboratory for various types of caries studies, the jaws have been carefully examined for the presence of gingival lesions. All such animals received a cariogenic diet over periods ranging from three to seven months. The diets varied in percentage content of certain items, but all were high in carbohydrate and all were

Fig. 5.

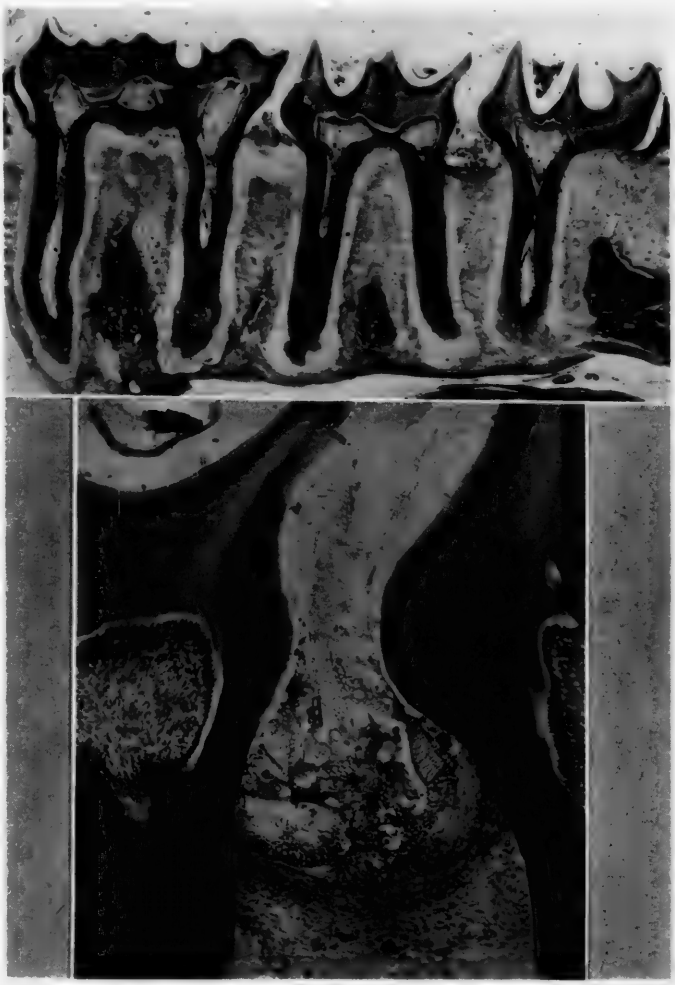


Fig. 6.

Fig. 5.—Mesiodistal section of lower molars (12-weeks). First molar at left. Normal gingivae between first and second molars, and beginning disease between second and third molars. Note height of alveolar crests of bone, width of periodontal membrane, and secondary cementum. (×22 orig. magnification.)

Fig. 6.—Interproximal area of destruction. Note accumulation of debris and underlying inflammation. Osteoclasts are present at crest of alveolar bone. (×100 orig. magnification.)

of a soft powdery consistency.* As considered, the animals belonged to two groups: (1) those which were housed with shavings, fifty-six animals; and (2) those which were housed on wire screen bottom cages (nominal opening, about, one-half inch square) without bedding, sixty-six animals.

Examination under magnifications up to ×30 revealed the fact that there frequently was a marked accumulation of a white, caseous material which resembled soft calculus in the gingival crevices. This was grossly apparent as a broad white-grey line around the necks of the teeth. Group I animals presented more normal gingivae with fewer and less extensive crevicular deposits than did those of group II. It should be noted that the animals of the two groups were not litter-mates, nor were the experimental periods of identical length.

Fig. 7 illustrates the microscopic findings in sections from an animal raised under these general conditions without bedding. Masses of basophilic material may be seen in periodontal pockets, with underlying inflammation and loss of alveolar bone.

Fig. 7.—Buccolingual section of upper second molars of animal with induced periodontal disease. Note accentuated gingival crevices and accumulation of basophilic material in this area. (Hematoxylin and eosin stain, ×15 orig. magnification.)

DISCUSSION

Considered only in the light of basic principles, a comparison between the dentitions of the human being and the hamster becomes reasonable, as far as the molars are concerned. Eruption, attrition, caries, periodontal disease, et cetera are processes common to both species, and the fundamental morphological characteristics are similar.

It appears that the incidence of that form of periodontal disease characterized by subgingival accumulations of hair and shavings increases with age in hamsters maintained under stock conditions. In only a few isolated instances were localized areas of inflammation visible grossly in the animals seven months of age and less.

*Example: Sucrose (4× confectionery sugar) 15 per cent; corn starch, 20 per cent; whole powdered milk, 30 per cent; whole wheat flour, 30 per cent; alfalfa, 4 per cent, and NaCl, 1 per cent.

The etiological mechanism involved in the induced type of gingival disease described would seem to be associated with the presence of a fine-ground, high-carbohydrate diet. Such a ration, with particular reference to the powdered milk therein, forms a sticky paste when moistened. This characteristic may be of significance in the production of the crevicular deposits. One or both of the following factors may be responsible for the seeming increase in incidence among those animals raised on the screen bottom cages: (1) the absence of wood shavings, which the animals chew, and which may serve as a gingival stimulator or dental and peridental cleanser, as suggested by King[9] with regard to periodontal lesion production in the ferret and/or (2) the inability of the animals to retain all their feces in the screen bottom cages, and thus the partial restriction of coprophagy, which may result in some nutritional deficiency.[10] To my knowledge, there have been no reports on this subject with reference to the hamster. Further studies are in progress.

Perhaps the chief significance of these findings rests on the fact that a simple means of inducing periodontal disease in large numbers of young animals is at hand. This set of conditions and the resulting predisposition to disease may facilitate the study of local and systemic factors related to the etiology, prevention, and therapy of various forms of periodontal disease.

SUMMARY

A brief description is given of the normal and diseased periodontium of the hamster. A form of periodontal disease common among aged stock animals and weanlings is described, and a set of conditions which appears to produce a unique type of disease in otherwise normal young animals is discussed. The hamster is recommended as a tool for research in the field of periodontia.

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ELECTROCHEMICAL IMPULSE THEORY OF DENTAL PAIN

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THERE are many observable phenomena associated with dental pain which may be explained by the electrochemical theory of dental pain. This theory is a collection of principles intended to explain clinical observations and, also, why and how pain is transmitted through a tooth. It is hoped that through application of the principles of the electrochemical theory, pain produced during cavity preparation may be eliminated. Theories that have been presented fail to explain the transmission of pain through enamel and dentin, for instance, when the tooth is so sensitive that the slightest touch of an explorer or a blast of air on the enamel causes pain.

A series of experiments was devised in development of the electrochemical theory.

EXPERIMENTAL

1. Cavities were drilled into a tooth on two sides. Copper wires were cemented into the cavities and the ends of the wires were attached to a galvanometer. When the tooth was wetted with hydrochloric acid, it acted as a battery, the galvanometer needle being deflected two or more marks.

2. The roots were cut off at the cervical region of a tooth and an opening made into the occlusal surface and into the root canal with a 560 cross-cut fissure bur. Copper wires were inserted into the holes and the ends of the wires attached to a galvanometer. When the tooth was wetted with copper sulfate solution, the galvanometer indicator was deflected.

3. A solution for wetting the tooth was made of table salt and water, and under conditions similar to those in the first two experiments, the indicator of the galvanometer was deflected. When a revolving bur was placed on the tooth, in this and the previous experiments, the direction of deflection was reversed.

4. A tooth was soaked in dilute hydrochloric acid for twenty-four hours. The tooth surface was attached to the galvanometer. While the tooth was wet, the indicator deflected a few degrees; after drying, only a fraction of a degree. After drying for one hour, the crown was cut off and a hole cut through the occlusal to the dentin. When the galvanometer wires were inserted into the occlusal hole and onto the pulpal surface, the indicator swung from 3.5 to 5 marks.

5. A freshly extracted deciduous tooth with a vital pulp was connected to the galvanometer. With one wire on the edge of the pulp and one on sound dentin, the indicator deflected about one-half mark. When the second electrode was moved to decayed dentin, the indicator swung beyond the markings on the dial.

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6. When one electrode of the galvanometer was placed on a sensitive tooth and the other on the palate or tongue, a slight deflection was noted. When the first electrode was placed on metallic fillings, the needle was deflected in the opposite direction.

DISCUSSION

It was shown that the tooth tends to act as a battery when wet with various solutions. If a current is to travel through the dentin and not stay on the surface, the enamel and dentin must be conditioned to permit passage of the current through the enamel to the dentinal fibers and to the pulp. Primarily, the resistance of the enamel to the flow of current must be reduced. The working theory is that when a spot is saturated with an electrolyte it affords a path, similar to a wire, leading to the pulp.

Experiments four and five indicate that a charge passes through the odontoblastic processes more readily than it passes over the tooth surface when these processes are conditioned. In the mouth, the entire tooth may be conditioned to favor passage of electrochemical impulses. Experiment six indicates that dental caries conditions the tooth for electrochemical impulses.

Huber,[1] Hopewell-Smith,[2] Chase,[3] and Wannack[4] have shown that there is no nervous tissue in any part of a tooth excepting the pulp. How then, can an impulse reach the nerve receptor of a tooth if not conveyed by nerve tissue? To understand this theory of dental pain thoroughly, it is necessary to understand the factors having a bearing on the conduction of a current of electricity through a tooth. A tooth must be conditioned to be made sensitive. An electrolyte is the most essential factor. A second condition for the easy conduction of a pain impulse is large fibers or a greater number of small ones. Gabel[5] quoted Langmuir as stating that molecules may orient themselves into positions making them more capable of transmitting an electrical current. This is a third factor. If foregoing statements are true, then, the pain impulse is formed in the pulp, but the stimuli is of electrochemical origin in and on the body of a tooth unless the pulp is exposed or involved by infection. Since the pulp contains nervous tissue, it may receive excitation and transfer it to the central nervous system.

Fibers affected by an electrolyte produced by caries or foods cause a tooth to become sensitive. Blood is an electrolyte that normally does not transfer the properties of conduction of electricity into dentin. Foods and diseases possibly impart qualities to blood which are capable of being transferred from blood to the dentinal fibers in the tissue fluid. Whether the electrolyte is introduced into the teeth by way of the pulps or by way of the outer surfaces, those teeth become sensitive. Acids of caries usually involve small areas in the beginning. As soon as the enamel has absorbed enough electrolyte, the charge of electricity takes the path of least resistance. Instead of being over the surface of the tooth, it now is through the affected part of the tooth to the pulp. The greater the number of fibers affected, the greater will be the impulse received by the pulp. Until enamel is conditioned, it is a poor conductor of electricity.

SUMMARY

The electrochemical theory of dental pain proposes that pain is conducted as an electrochemical impulse through conditioned enamel and dentin to the pulp where nerve tissue is present. It can explain phenomena observed clinically and not explained by other concepts.

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PROCEEDINGS OF THE UNIVERSITY OF MICHIGAN SCHOOL OF PUBLIC HEALTH AND SCHOOL OF DENTISTRY INSERVICE TRAINING COURSE FOR THE EVALUATION OF DENTAL CARIES CONTROL TECHNICS

September 8 through 13, 1947

THURSDAY, SEPTEMBER 11

Morning Session

(Auditorium, School of Public Health)

DR. EASLICK.—My congratulations this morning to you "students," some of the professors, and some of the faculty. Some times yesterday afternoon I thought that the faculty was faltering, but I was amazed how the students carried on. I realize that it has been a long, long time since some of you dentists have found yourselves in a study situation. So, I am quite sincere in congratulating you on the amount of effort that you spent in our hot dental library yesterday afternoon. I have a feeling that all of the faculty should be available in the library this afternoon to help the "students" locate references that may answer their questions. We have an abundance of literature available, but when it comes to literature that will answer specifically a specific question, it is very, very scarce, indeed. From your store of knowledge of references you can often make a helpful suggestion to the people evaluating information. I am sure many of them would appreciate your suggestions very much.

We are continuing the study of fluorine further this morning, and we also shall have to stain up the morning with some silver nitrate. The first of the speakers this morning is Dr. John W. Knutson, who is chief of the Dental Section of the States Relations Division of the United States Public Health Service. The assignment which our group gave Dr. Knutson is "An Evaluation of the Effectiveness as a Caries Control Measure of the Topical Application of Solutions of Fluorides." With some notion about his discussion from previous opportunities that I had in Atlanta, Georgia, and Boston, Massachusetts, I know that this phase of caries control will be covered very well, indeed. I am giving him the first forty-five minutes this morning. Dr. Knutson.

AN EVALUATION OF THE EFFECTIVENESS AS A CARIES CONTROL MEASURE OF THE TOPICAL APPLICATION OF SOLUTIONS OF FLUORIDES

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My premiere performance on this very same subject was given about five weeks ago at Boston. At that time the conclusion was that the use of topical fluorides as a preventive measure in dental caries control is approximately 40 per cent effective in reducing the incidence of the disease. That is the conclusion we shall reach again, I believe, after we consider all of the evidence related to the subject: the chemical evidence, the evidence resulting from laboratory investigations, the clinical evidence, and also what has been referred to here as evidence that is of concern from a logical standpoint.

In presenting the various categories of evidence, I shall not pay particular attention to the chronology of published reports. Rather, I shall attempt to present them according to whether or not they are chemical evidence, animal laboratory evidence, or clinical evidence on human beings, and to include related evidence which at the time the observations were made may not have been considered to be evidence which would be of any concern to this subject, that is, the use of topical fluorides as a caries preventive.

Shortly after it was determined that fluorides were the cause of mottled enamel, a concerted effort was made by a few individuals to discover, or find, an agent that might be used for removing excessive fluorides from drinking water supplies. One of the substances found to be effective was bone meal. Later, several investigators Elvove,[1] MacIntire and Hammond,[2] Adler, Klein, and Lindsay[3] found that the principal constituent of bone meal that appeared to be—or that was—the effective agent for the removal of fluorides was tricalcium phosphate, one of the principal components of tooth substance.

At the time Armstrong and Brekhus[4] discovered that enamel of sound teeth contained more fluorine than enamel of carious teeth, there probably wasn't much thought that this constituent of enamel could be increased by direct application. Certainly prior to this discovery it was generally accepted, perhaps more by implication than otherwise, that once enamel had erupted into the mouth it could not be changed constructively. When it was definitely determined that there was a specific relationship between fluorides in water supplies and dental caries, interest was stimulated in studying the manner of action of the fluorides and the manner of acquisition of the fluoride by enamel. These studies were principally chemical laboratory investigations. Perhaps the first of these of significance was that reported by Volker and his associates[5, 6] showing that the solubility of enamel could be appreciably reduced by treating it with a fluoride solution. They also found that enamel, bone, dentin, and hydroxyapatite, all these substances, would adsorb fluorine according to a definite pattern. Norvold, Inglis, and Armstrong[7] also reported evidence that enamel would or could adsorb fluorides.

The next chemical studies of concern (and, as you recognize, I am giving you merely the conclusions instead of the details of these related findings) were designed to determine what fluoride solution was absorbed at the greatest rate, or in the greatest amount, and what pH of the fluoride solution was the most effective.

Bibby[8,9] found that as the pH of the solution was lowered, the fluorides were adsorbed more effectively. He also found that of the several fluoride solutions tested, sodium, potassium, and ammonium appeared to have the same adsorption qualities, whereas calcium fluoride was ineffective. Phillips and Muhler[10] also showed that as the pH of the fluoride solution is lowered, the amount of fluoride adsorbed is increased. They concluded that the optimum pH was about 2.6, and that this pH was not low enough to damage tooth tissue.

Buonocore and Bibby[11] then conducted a series of studies to determine which of the several fluorides might be most effective, and from the results of their laboratory findings concluded that lead fluoride was appreciably more effective than sodium fluoride. Later, Muhler and Van Huysen[12] conducted studies of a similar nature and concluded that tin fluoride was the most effective. It is of interest that from the results of their studies, they arrived at the conclusion that lead fluoride and sodium fluoride had about the same adsorption qualities in so far as enamel was concerned.

With reference to the manner of acquisition of the fluoride or deposition of the fluoride in the tooth or in the enamel, my summary statement is that at present it is *not known* just how the fluoride is deposited or in what form it is deposited in the enamel. There are those who have studied this problem who believe it is deposited in the form of calcium fluoride; and others, that it is in the form of calcium fluorapatite, a direct adsorption compound formed as the result of the application of fluoride solution to enamel.[13-15]

Our next category of evidence will be concerned with laboratory investigations on animals. I might say that only a very limited amount of work on animals having a direct relationship to this subject has been done. Sognnaes[16-17] conducted two studies on small groups of rats and came to the conclusion that the evidence suggested that fluoride solutions, used topically, reduced dental caries incidence. Keyes[18] used hamsters for his experimental animals and arrived at a similar conclusion. His findings in a small group of hamsters were not conclusive, but the data indicated that there was a reduction in caries incidence when the teeth were treated with topical sodium fluoride.

The next subject, and perhaps the most important feature of this evaluation, will be a presentation of clinical evidence on human beings. As Dr. Wallace pointed out to you, in many dental investigations the clinical evidence is the clinching feature because we do not fully understand the carious process. In our evaluation of this evidence (Table I) we shall be concerned with numerous variables. The important variables are: the number of cases in the study groups, the method of control and of treatment, the technic of application, the age of the group, the fluoride solution and its concentration, and dental prophylaxis. Dental prophylaxis is an incorrect term as used here.

It is really not a prophylaxis such as was described to you by Dr. Hine.* We did not use the traditional dental prophylaxis, but what we did was remove the materia alba from the teeth. Perhaps a better term for this is the one used by Dr. Fosdick. We just took the "gunk" off the teeth. We merely used a rubber cup and fine pumice to cleanse the teeth, a procedure that requires about five minutes, not any more than an average of five minutes. With few exceptions, the children with whom we have been working don't have calculus, but the use of the rubber cup with fine pumice is very effective in removing the gross debris in a very short time.

One of the variables that does not receive its share of attention from dental investigators is the number of children studied. In order that a statement made by Dr. Wallace, and concurred in more or less by Dr. Fosdick, is not misinterpreted, I might point out one important thing to consider in terms of numbers in setting up studies, namely, that you are usually concerned with two variables or sources of error in your study groups. One is error in measurement, the error in the tools that you use, or the variability in application of the devices used in measurement. The second is the individual variability within the group. Usually, dental investigators are very much concerned about the error of measurement, they will report how carefully and in how much detail each case was studied, that x-rays were made, that transillumination was used, and that fifteen to twenty minutes were required to complete each examination of the nine, twenty-one, or twenty-seven cases in the study group. Regardless of what is done to reduce the error of measurement, the real range of variability among individuals within the group is not affected. To illustrate, let us say that the height of the freshman students at the University of Minnesota is measured very carefully so that the error of measurement is reduced to a small fraction of an inch. The care exercised in making the measurements has not reduced the range of variability within the group, which might be as much as 24 inches. In general, it is only by having a large enough number of cases in our study groups that we eliminate chance variability within the groups as the cause for observed differences.

Now, I do not want to give the impression that we should not be concerned with using a careful and standard procedure for obtaining our measurements. The same procedure should be used on the control as on the experimental group. The procedure is important, we want to have a standard technic so that we examine, let us say, the experimental and the control children in the same way. But we must have enough individuals in each group so that differences effected by the application of a preventive are not obscured by individual variability, or, on the other hand, so that differences due to individual variability are not attributed to an ineffective preventive agent.

This point perhaps can be even better illustrated by an example of an exaggerated situation. In 1936, a newly discovered preventive for poliomyelitis was tested with 500 children treated in an experimental group and 500 children

*Hine, M. K.: Prophylaxis, Toothbrushing, and Home Care of the Mouth, *J. D. Res.* 27: 223, 1948.

retained in a control group. After the polio season was over, an analysis was made by two investigators[19] to see how effective the vaccine had been. With the experiment completed, they found that if the difference between the incidence of polio in treated and untreated children had been 80 per cent, they would have needed 27,000 cases instead of 1,000 in the two groups. If the vaccine had been 100 per cent effective, they would have needed 15,000 cases in the two groups to show that the difference was significant. Now, I say I am using an exaggerated case; the incidence of polio is much lower, fortunately, than that of dental caries, but by and large, the same principle applies.

We can estimate before we start a clinical study for testing a caries preventive how many cases are needed with an attack rate—an estimated attack rate—of, say, 10 per cent per year among the teeth, or only 5 per cent. We can estimate how many individuals are needed in the sample to measure the significance of a difference in caries rates between treated and untreated teeth. Therefore, sample size is always an important item to watch when we evaluate the results of clinical studies in dental caries control.

Bibby[20] (Table I) treated the teeth in one quadrant in the mouth of each of ninety individuals and used the teeth in the untreated quadrants as controls. He used a 0.1 per cent sodium fluoride solution and made three applications during the year, spaced three to four months apart. One year after the initial application, he found 45 per cent less new caries in the fluoride-treated teeth than in the untreated teeth. Two years after, the treatment was continued to a total of six treatments at the rate of three treatments a year. The difference was 33.3 per cent. Three years after treatment was completed, or five years after the initial application, there was 36 per cent less caries in the treated than in the untreated teeth.

Knutson and Armstrong[21-23] (Table I), in a study of 289 children, treated the teeth in one side of the mouth and used the teeth in the untreated half of the mouth for controls. These investigators applied a 2 per cent sodium fluoride solution to a total of seven to fifteen applications. All applications were made during an eight-week period, at a rate of one to two treatments per week. During the year following treatment, the incidence of dental caries was 40 per cent less in the fluoride-treated than in the untreated teeth. For the first two-year period, the difference was 41 per cent, and for three years, 37 per cent less new caries in the fluoride-treated teeth than in the untreated teeth. An internal analysis of the findings for this study group indicated that eight applications effected a reduction in caries incidence equal to that of fifteen applications, leading to the conclusion that fifteen applications were not more effective than eight.

Jordan and his associates[24] (Table I), using the same technic as was used in our first study, were interested in determining the minimum number of treatments which would give maximum effectiveness. With one application, 240 individuals demonstrated 5 per cent reduction; almost 600 individuals given two applications had 10 per cent less new caries; 160 individuals given three applications had 21 per cent reduction.

TABLE I. STUDIES OF TOPICAL FLUORIDES

STUDY	NUMBER TREATED	TEETH TREATED	CONTROLS	AGE	PROPHY.	SOLUTION	NUMBER OF APPLICATIONS	YEARS SINCE FIRST APPLICATION	PER CENT REDUCTION
Bibby	90	One mouth quadrant	Untreated mouth quadrant	10-12	Yes	.1% NaF	3	1	45.8
	78	One mouth quadrant	Untreated mouth quadrant	10-12	Yes	.1% NaF	6	2	33.3
	39	One mouth quadrant	Untreated mouth quadrant	10-12	Yes	.1% NaF	6	5	36.0
Knutson and Armstrong	289	One side of mouth	Untreated half of mouth	7-15	Yes	2% NaF	8-15	1	39.8
	270	One side of mouth	Untreated half of mouth	7-15	Yes	2% NaF	8-15	2	41.4
	242	One side of mouth	Untreated half of mouth	7-15	Yes	2% NaF	8-15	3	36.7
Jordan, Wood, Allison, and Irwin	241	One side of mouth	Untreated half of mouth	6-12	Yes	2% NaF	1	1	4.9
	575	One side of mouth	Untreated half of mouth	6-12	Yes	2% NaF	2	1	10.0
	161	One side of mouth	Untreated half of mouth	6-12	Yes	2% NaF	3	1	21.0

TABLE II. STUDIES OF TOPICAL FLUORIDES

STUDY	NUMBER TREATED	TEETH TREATED	CONTROLS	AGE	PROPHY.	SOLUTION	NUMBER OF APPLICATIONS	YEARS SINCE FIRST APPLICATION	PER CENT REDUCTION
Knutson, Armstrong, and Feldman	472	One side of mouth	Untreated half of mouth	7-15	No	2% NaF	2	2	9.3
	504	One side of mouth	Untreated half of mouth	7-15	No	2% NaF	4	2	20.1
	482	One side of mouth	Untreated half of mouth	7-15	No	2% NaF	6	2	21.0
Galagan and Knutson	301	One side of mouth	Untreated half of mouth	7-15	Yes	2% NaF	2	1	21.7
	247	One side of mouth	Untreated half of mouth	7-15	Yes	2% NaF	4	1	40.7
	259	One side of mouth	Untreated half of mouth	7-15	Yes	2% NaF	6	1	41.0
	272	One side of mouth	Untreated half of mouth	7-15	Yes	.06% PbF_2	2	1	5.9
	214	One side of mouth	Untreated half of mouth	7-15	Yes	.06% PbF_2	4	1	1.6
	262	One side of mouth	Untreated half of mouth	7-15	Yes	.06% PbF_2	6	1	0.8

The technic used in the study by Knutson, Armstrong, and Feldman[25] (Table II), was the same as that used in the first study by Knutson and Armstrong, except that we did not clean the teeth before the fluoride solution was applied. Note that the number of cases in each of these groups is quite large. With two applications without a cleansing—an initial cleansing—there was 9 per cent reduction; with four applications, 20 per cent reduction; with six applications, 21 per cent reduction. I want to pause here to point out that this, together with the other studies (our first studies, the studies by Jordan and these), suggests that the maximum number of treatments is between four and six, or might be four. Six did not give more effective results than four.

In our most recent study, that by Galagan and Knutson [26] (Table II), the same procedure was used, except that prior cleansing of the teeth was included. They were cleansed before the series of either two, four, or six applications. See what happens? Two applications yield 21.7 per cent reduction, about twice as much as without the cleansing; four applications, 41 per cent, about twice as much as without the cleansing; six applications, 41 per cent, no difference between the last two, and again twice as much as without the cleansing. The conclusion from this series of studies is that four applications of a 2 per cent solution of sodium fluoride, in which the initial application is preceded by a cleansing, will effect a 40 per cent reduction in dental caries.

I pointed out that some of the chemical studies indicated that there were other fluorides that might be more effective than sodium fluoride, and I can assure you that studies will be continued to find a more effective fluoride solution than sodium fluoride, to find a simpler and more effective treatment method, but as of today four applications of a sodium fluoride solution give us the maximum result when a 2 per cent solution is used.

We tested clinically one fluoride solution that from laboratory findings was supposed to be more effective than sodium fluoride, according to one investigator, and equally as effective as sodium fluoride, according to another laboratory investigator. In other words, we repeated our usual clinical study using lead fluoride instead of sodium fluoride (Table II). By and large, the answer here is that lead fluoride is not effective at all, at least in the manner in which we used it for reducing dental caries incidence.[26]

I want you to continue to watch the number of cases. Cheyne[27] (Table III) had twenty-seven cases, children 4½ to 6 years of age, in his treated group and nineteen control cases, and he worked with deciduous teeth. He treated the teeth with a .05 per cent potassium fluoride solution to a total of two applications and reported 50 per cent reduction. His study was over a period of slightly more than three months. Jordan and co-workers[24] (Table III, which presents the results in the deciduous teeth of the group for whom we previously presented the results in the permanent teeth)—obtained reductions in caries incidence with one, two, and three applications: reduction of 5 per cent with 1; 15 per cent with 2; and 40 per cent with three. Because of the ages of the individuals in these groups, they are not ideal for studying the effect of topical fluorides on deciduous teeth. In other words, in the older age ranges the children do not have many deciduous teeth.

TABLE III. STUDIES OF TOPICAL FLUORIDES

STUDY	NUMBER TREATED	TEETH TREATED	CONTROLS	AGE	PROPHY.	SOLUTION	NUMBER OF APPLICATIONS	YEARS SINCE FIRST APPLICATION	PER CENT REDUCTION
Cheyne	27	All teeth (deciduous)	19	4½-6	Yes	.05% KF	2	.28	50
Jordan, Wood,	241	One side of mouth (deciduous)	Untreated half of mouth	6-12	Yes	2% NaF	1	1	4.9
Allison, and	575	One side of mouth (deciduous)	Untreated half of mouth	6-12	Yes	2% NaF	2	1	14.5
Irwin	161	One side of mouth (deciduous)	Untreated half of mouth	6-12	Yes	2% NaF	3	1	40.0
Arnold, Dean, and Singleton, Jr.	94	All teeth	94	16-23	Yes	.5% NaF	1	1	0
East, Ziskin, Stowe, Karshan, and Richardson	48	One tooth, per child	48	Unk.	No	.05% KF	1-4	1	0
McCauley and Dale	21	One side of mouth	Untreated half of mouth	2-13	No	.1% NaF	3-11 (Average, 7.4)	1	Bilateral
	21	One side of mouth	Untreated half of mouth	2-13	No	.1% NaF	3-11 (Average, 7.4)	2	0

Arnold and associates[28] (Table III), had Coast Guard cadets, 16 to 23 years of age, who received one application of a 1 per cent sodium fluoride solution, acidulated solution, and ninety-four paired controls that were studied for a period of one year after treatment. They observed no reduction in dental caries. This finding may or may not be in accord with other findings on topical fluorides. Jordan and associates got a 5 per cent reduction with one application. To establish a reduction of five per cent as statistically significant would require even greater numbers than either Arnold and co-workers or Jordan and co-workers had in their study groups.

East and his associates[29] (Table III) treated forty-eight individuals, but they treated only one tooth in each child and used the homologous tooth as a control. Therefore, they treated a total of forty-eight teeth, and concluded that their results were negative. The treatment was not preceded by a prophylaxis. I might point out that they reported that there were twelve new decayed teeth in one set of these forty-eight teeth and fifteen in the other. They didn't identify treated and control groups in the report, but if the twelve were in the fluoride-treated group and fifteen in the untreated group (although much reliance cannot be placed on such limited experience when dealing with dental caries), that would mean a 20 per cent reduction, which is in accord with the reduction effected without prior cleansing of the teeth.

McCauley and Dale[30] (Table III) treated the teeth of twenty-one children. They treated the teeth in half of the mouth; they did not clean the teeth and they did not isolate the teeth. After the first year of study they concluded that they got a bilateral reduction in dental caries. The caries rate for this study group for the first year was lower than that expected, but it was bilaterally equal, and so they concluded that they got a bilateral reduction. They followed the group for another year and found that the rate for each of the two halves of the mouth had increased markedly for the year, and so they concluded that for the two-year period their results were negative. Now, not only is the number of cases in this study small, but the treatment procedure varies widely from the treatment procedure that was discussed earlier. They did not clean the teeth, they did not isolate the teeth, and they did not dry the teeth before the fluoride solution was applied.

The procedure that we used to effect a 40 per cent reduction in dental caries was to cleanse the teeth (a five-minute cleansing, or the average of a five-minute cleansing per child), isolate the teeth with cotton rolls, dry them with compressed air, wet the crown surface of the teeth with 2 per cent sodium fluoride solution, and let the applied solution dry in air.

Tables IV and V are presented merely to point up the method used in conducting these studies and the results. Table IV illustrates the details of what had occurred during the two years since the topical fluoride treatments were administered to half the teeth of 289 children, 270 of whom were available two years after treatment.[22] This study was based on the observation, which has been repeatedly confirmed, that the occurrence of dental caries is equal bilaterally in population groups. In the fluoride-treated upper teeth during the

two-year period, there were ninety-seven new decayed teeth and one hundred seventy-four in the untreated teeth. There is certainly no bilateral similarity there. Of the lower teeth, sixty-seven decayed in the fluoride-treated teeth, 107 in the untreated teeth, and there is certainly no bilateral occurrence there.

TABLE IV. DENTAL CARIES INCIDENCE IN SODIUM FLUORIDE-TREATED AND UNTREATED PERMANENT TEETH. FOR TWO-YEAR PERIOD ENDING MAY, 1944; 270 MINNESOTA CHILDREN, 7 TO 15 YEARS OF AGE

QUADRANT	NUMBER NONCARIOUS TEETH (APRIL, 1942)	NUMBER BECOMING NEWLY CARIOUS (TWO-YEAR PERIOD ENDING MAY, 1944)
	Upper	
Treated (left)	929	97
Untreated (right)	940	173
	Lower	
Treated (left)	1,145	67
Untreated (right)	1,154	107

In our first study, we had a negative control group so that we could compare caries experience in the untreated half of the mouth of the experimental group with that of children who had similar caries experience but who had received no treatment whatever except the cleansing of the teeth. Table V illustrates what happened in the control group during the two study years: 253 new decayed teeth in the upper left; 254 in the upper right; lower, 137 on the left and 137 on the right. That is almost perfect bilateral symmetry. I can assure you that it does not always come out that way, even with so large a number of individuals in the study groups.

TABLE V. DENTAL CARIES INCIDENCE IN PERMANENT TEETH, BY LEFT AND RIGHT MOUTH QUADRANTS; FOR TWO-YEAR PERIOD ENDING MAY, 1944; 320 MINNESOTA CHILDREN, 7 TO 15 YEARS OF AGE (CONTROL GROUP)

QUADRANT	NUMBER NONCARIOUS TEETH (APRIL, 1942)	NUMBER BECOMING NEWLY CARIOUS (TWO-YEAR PERIOD ENDING MAY, 1944)
	Upper	
Left	1,322	253
Right	1,317	254
	Lower	
Left	1,465	137
Right	1,489	137

SUMMARY

In summary review, chemical studies were conducted to test the effectiveness of different fluoride solutions. These studies indicated that there may be fluoride solutions more effective than sodium fluoride. I hope such solutions are found. However, until such solutions are tested clinically, it is strongly indicated from the results of the tests with lead fluoride that we consider the use of any other fluoride solutions than sodium fluoride as experimental.

Consideration has been given in the laboratory to the difference in pH level of the solutions used. The conclusion was reached by one group of investigators that a pH as low as 2.6 would be noninjurious to the tooth tissue.

I, myself, am not ready to accept that conclusion. I think this matter deserves further study, particularly in view of the fact that it has been demonstrated that decalcification can occur and does occur at a pH of 5, and, certainly, definitely at a pH of 4. Studies are now being conducted that should shed additional light on pH very shortly. Some of these studies are concerned with tooth surface patterns or tooth surfaces as seen through the electron microscope. Until it has been verified, I do not think I would use a solution with a pH as low as 2.6. As a matter of fact, I do not think the pH should be varied in any way until verified by clinical studies.

There was great variation in the spacing of the applications. Some of the investigators gave three applications a year, spaced four months apart. In all our studies, we gave the applications at a minimum rate of one a week, and a maximum rate of two a week to whatever total number was desired. It is merely my impression that if four applications do give the most effective results and four are required to give the full effects of the topical fluoride that we elect to use, it seems to me that the earlier we get those on the teeth, the better. It is also very likely that if you do space the treatments far apart, it would be important to precede each application with a cleansing.

Concentration of the solutions used varied widely. Most of the work has been done with 2 per cent sodium fluoride solution. Indications are that a 1 per cent solution may be fully as effective, but again this concentration has not been tested as fully as the 2 per cent solution.

One concluding item of interest is the effect of topical fluorides on lactobacillus counts. When we treated children's teeth, that is, the full mouth, and studied the lactobacillus acidophilus counts, we got no reduction in the lactobacillus acidophilus counts. Now, that can be explained, I am quite sure, and really does not reflect on the effectiveness or the usefulness of lactobacillus acidophilus counts. In our first study on this particular phase, we had twenty-five children in each group, both control and experimental. We made lactobacillus acidophilus counts at intervals of three months, and there was no change during the year. We followed a similar procedure with another study of forty children in each group. Again, we made lactobacillus acidophilus counts, but at time intervals of one year, and for a period of two years we got no change in the counts.

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DISCUSSION

DR. EASLICK.—Thanks very much, Dr. Knutson. Dr. Knutson's report is open for discussion or questions.

DR. ALLEN GRUEBBEL (American Dental Association).—I should like to ask Dr. Knutson if he would expect a greater reduction in caries incidence if each application of the fluoride solution were preceded by a cleansing of the teeth.

DR. KNUTSON.—That is a very important question; at least, it is a good question for a good deal of conjecture. We have tried to analyze our figures by internal comparisons to determine the rationale of cleansing. It seems reasonable to believe that the teeth surfaces should be cleaned before the fluoride solution is applied if maximum action is to be facilitated. It is of interest, however, that in terms of percentage caries reduction, the first application, which is the one immediately preceded by a cleansing, is not more effective than the second or fourth. Whether or not these results depreciate the importance of cleansing before the second, third, or fourth applications, I don't know. For a definite answer to your question we must await the results of clinical trials.

DR. EASLICK.—Dr. Grant has the floor.

DR. EDMUND A. GRANT (Department of Public Health, Toronto, Ontario).—Dr. Easlick, I should like to ask Dr. Knutson a question. I came across a new word the other day. I should like to take advantage of this opportunity to use it, and so I should like to ask Dr. Knutson if fluorides as used in the topical application technic are "absorbed" or "adsorbed." I should also like to ask him whether any precautions were taken to prevent the child from swallowing the solution, or if he believes the 2 per cent solution is toxic when swallowed.

DR. KNUTSON.—There is a difference between absorption and adsorption, but it has been my feeling in recent years that when we don't know whether a substance is absorbed or adsorbed, we use the term adsorption. We don't know whether the fluoride is absorbed or adsorbed by enamel.

With reference to your question on the toxicity of the fluoride solutions used, it should be clear that we are dealing with a toxic substance, but that the amount used in the topical fluoride procedure is not toxic. One would have to drink from six to eight ounces of a 2 per cent sodium fluoride solution to get serious toxic effects. Therefore, we don't require a rinsing of the patient's mouth with water after the treatment has been given.

DR. EASLICK: Dr. Strusser, I think you had your hand up?

DR. HARRY STRUSSER (Bureau of Dentistry, New York City).—I should like to ask a question, but first I should like to have Slide two [Table II] put on.

Since Dr. Knutson has made a point of the prophylaxis and then has changed it to a cleansing to remove or thin down the materia alba, or whatever material is on the surface of the tooth, would it be possible to get a better result with the use of a prophylaxis and a porte-polisher so that material is removed much more effectively from the tooth surfaces, and so that the penetration of the sodium fluoride might be greater and thereby effect a higher percentage caries reduction in areas which are susceptible and which are not reached by the cleansing alone?

DR. KNUTSON.—I don't know, but it is possible. One possibility that sounds better to me right now, after hearing Dr. Kesel's paper, is the use of his mouthwash prior to application to remove the materia alba.

DR. STRUSSER.—May I continue? It seems that these results, then, depend upon penetration and the thickness of that materia alba, and if we can remove it or thin it down as much as possible, we might get a better and greater absorption of the sodium fluoride.

DR. KNUTSON: Yes, I agree, that is a possibility.

DR. EASLICK: Dr. Atkins, did you want the floor?

DR. PAUL ATKINS (Des Moines, Iowa).—I am a practicing dentist, and, as you know, we have been cautioned by statisticians to evaluate data very carefully. I should like to know if Dr. Knutson uses a rubber dam and if any of the fluoride solution is carried over to the untreated control side of the mouth. I should also like to ask if sufficient numbers were used from which to draw definite conclusions.

DR. KNUTSON.—Yes, sir, I think the numbers are large enough. As I showed you in our negative control group, which we followed for the first two years with our first study group, the caries was bilaterally equal. The rate of caries in each half of the mouth of the controls was the same as in the control teeth of the experimental group. We didn't use the rubber dam. We isolated the teeth with cotton rolls, dried the teeth thoroughly, and applied the fluoride solution. The fluoride solution was permitted to dry on the teeth about three minutes before the patient was dismissed.

DR. ATKINS.—Do you think it is, as we say, adsorbed? The saliva doesn't come along and wash some over onto your nontreated side? Do you feel that in the saliva there is an insufficient quantity of the solution?

DR. KNUTSON.—It is possible, but it seems to me the concentration of that solution might be very low, and, further, that it would be dissipated in the saliva and swallowed, or expectorated. There is a possibility of getting that effect. It didn't show up in our studies. The control half of the experimental group had as much caries as either half of the mouths of, say, the negative control group. There is some suggestion in a recent study that we may be getting a slight reduction in the control half of the mouth also. I hope that is true, in which case over 40 per cent is a conservative figure.

DR. ATKINS.—That is the main point I wanted to bring out: You are getting benefit on both sides, and your comparison with your control group is more justified than your two halves of the mouth.

DR. HAROLD J. NOYES (University of Oregon Dental School).—Dr. Knutson, I should like to ask if an analysis has been made of the location of the lesions which appear in the control and the treated side of the mouth?

DR. KNUTSON.—In answer to your question, I might say we were interested in that early in this study because it was pointed out that in areas where fluoride is normally in the water supply one part per million and higher concentrations, the reduction in caries is greater in the anterior teeth. So, we tried to determine where the caries was reduced in this group, but the numbers were not large enough to reflect any significant results. Some of our most recent findings have not been published yet, but I think we have sufficient data now to get the answer to your question. We don't have it at present.

DR. NOYES.—I am not only thinking of teeth; I am thinking of surface areas.

DR. KNUTSON.—That is right, we have the records that can be analyzed. In our first analysis there was so much variability that conclusions did not seem warranted.

DR. NOYES.—It might have a bearing on whether or not it is significant to go into interproximal spaces in considering the extent of your prophylaxis.

DR. GENEVIEVE STEARNS (University Hospital, Iowa City, Iowa).— Dr. Knutson, to go back to the significance of the difference, do you remember, offhand, your standard deviations in the control and experimental group? How wide were those variations?

DR. KNUTSON.—We made a thoroughly statistical analysis of the data for the first study group, at which time we were concerned with answering the question, "Can you affect caries incidence with topical fluoride solutions?" That was four years ago, and I don't recall what the standard deviation was, but certainly the difference between the treated and the control groups, by several methods of testing the data, was statistically significant.

DR. STEARNS.—I am sure that it was. I merely wanted to know, what was the range of variation that you got among the children of each group in your standard deviation?

DR. KNUTSON.—In the amount of new caries?

DR. STEARNS.—Yes.

DR. KNUTSON.—I don't recall what that was.

DR. DONALD A. WALLACE (American Dental Association).—In relation to the toxicity of the 2 per cent solution, I believe you said that it would take six to eight ounces to produce a serious effect. I don't recall precisely what the recorded toxic and fatal doses are in the formal college text, but I believe five grams is considered a fatal dose of sodium fluoride, and that is a little more than would be present in six or eight ounces of a 2 per cent solution. Lest anybody be careless in his handling of the 2 per cent solution, I think it ought to be understood, if I am correct, that a 2 per cent solution, if it is swallowed in any quantity, is dangerous, but the amount that you applied in your clinical work probably is not hazardous.

DR. KNUTSON.—Thank you, I think that is right.

DR. HAMILTON B. G. ROBINSON (Ohio State University).—This prophylaxis or cleansing has intrigued me for some time, and I can't understand the significance of it. If you give a prophylaxis to a tooth and then you extract it, stain it, and examine it, you will find that with a rubber cup, or probably with even a porte-polisher, you have not removed the plaque material or the materia alba, or whatever you want to call it, down in the fissure or just beneath the contact point where caries will start. I wondered if you had investigated the possibility that if you removed the materia alba because part of the fluorine, a large part of it, might be absorbed into the materia alba and reduce its effectiveness in the quantity that would reach the tooth. The explanation of just removing material and letting it get into the tooth wouldn't hold up very well.

DR. KNUTSON.—I have been as concerned about those things, I think, as you have, Dr. Robinson. In fact, there are several things that don't appear quite logical in this

whole procedure. For instance, one of the things that we have wondered about is, why can't one with a thorough treatment the first time get as much absorption as one does with four treatments or applications? There is no evidence that that is true. I am sorry, but I can't answer your question on the basis of information now available.

DR. PHILIP E. BLACKERBY (W. K. Kellogg Foundation).—Dr. Knutson, are you prepared to make a recommendation regarding the use of cotton swabs, an atomizer, or other means for the application of the fluorine to the tooth?

DR. KNUTSON.—Either might be used, I think, with equal effectiveness. It depends upon the individual's desires. We have used both. In most of our work we used the cotton applicator, but the usual cotton applicator has twice too much cotton on it to be handled readily. If you can control an atomizer and control the pressure on your unit so that you get it on the tooth and don't use it too liberally, that method is also effective. The main thing is to wet the surfaces of the teeth thoroughly. I am not prepared to say that one method, either the cotton applicator or the atomizer, is more effective or more efficient.

DR. EASLICK.—Dr. Hoffman, you requested opportunity to ask a question?

DR. OLIN HOFFMAN (Iowa State Department of Health).—Yesterday I heard someone say that Dr. Knutson said that you could drink six or eight ounces of sodium fluoride without any danger. Now, you see how that information is distorted as it gets around. I believe that Gould's Medical Dictionary shows that four grams of sodium fluoride constitutes a fatal dose, and I think Dorland states that ten grams constitutes a lethal dose, so that if you actually drank eight ounces of 2 per cent solution you would be getting ten grams, or maybe twice the amount that would be necessary to kill a human being.

DR. KNUTSON.—I just want to say that I am glad that point was emphasized, and particularly as Dr. Wallace brought out, that you are dealing with a toxic substance and that there are dangers involved. On the other hand, it may be important that the 2 per cent solution is quite salty in taste, and it is not likely that individuals would drink it, certainly not with pleasure.

DR. ROBERT M. STEPHAN (United States Public Health Service).—I should like to comment on this cleansing effect. In some of our studies on the penetration of detergents and substances through plaque material, I think we showed that it is rather difficult for many diffusible substances to get through; that is, it takes them considerable time. Therefore, with the cleaning of teeth, it is logical to expect an increase of effectiveness. On the other hand, of course, if there were material on the teeth, it would tend to retain on the teeth any fluoride that had been taken up by that material. You would have a double effect, and, therefore, I think you can't assume that you could vary Dr. Knutson's procedure, or the procedures used in cleaning the teeth. In order to get the same results, it may be necessary to have two effects.

DR. J. M. WISAN (New Jersey State Department of Health).—I should like to ask this practical question that seems to be causing controversy, as to whether the dental profession should make the application, or whether the auxiliary personnel might apply the solution.

DR. KNUTSON.—I think from a public health standpoint your question is very important, Dr. Wisan, and the only answer I can give is one based on my own experience, in which we used a dental hygienist to assist in making the applications. It is my impression that she did the job equally as well as I did. In talking about the use of auxiliary aides,

I think that it is important to determine whether or not we shall reduce the standards of our methods of procedure by doing so. In this case, I am convinced that we shall not. A dental hygienist can treat approximately 3,500 children a year with a four-treatment series and using the method that I have described, at a cost slightly below a dollar per series of treatments per child. Perhaps the minimum number of treatments for a child should be about four complete series of four applications each. You would want to treat the deciduous teeth, let's say, at the age of 3 years. Since the effects are retained, in so far as we know, they would not have to receive additional treatments, but you want to treat the teeth during the period of changing dentition, from the ages of 6 to 12 years. You might give a series of treatments at ages 3, 7, 10, and 13 years. At the age of 13, you would get all the permanent teeth excepting the third molars. Now, we all recognize that if this work were to be done by dentists, it would cost appreciably more, just how much more I am not prepared to say. Others may have figured out what that cost would be if the dentist did the work himself. Certainly every dentist who renders treatment for children should use topical fluorides and charge a fee commensurate with other fees charged for his service time.

DR. WISAN.—Would you please name those suggestive ages again?

DR. KNUTSON.—3, 7, 10, and 13 years.

DR. EASLICK.—Dr. Medof, did you have a question?

DR. BENJAMIN M. MEDOF (Minneapolis, Minn.).—Yes, I might have missed a point in listening to Dr. Knutson. I wondered how long he leaves the application on one side before he goes to the other. Another question, I should like to know, is there any evidence of fluorine being found on the inside of the enamel?

DR. KNUTSON.—After the fluoride solution is applied, it is permitted to dry in air, which takes about three minutes. I might add that in my estimate of the number of children a dental hygienist can treat, she has three or four children seated in a row at one time. While the fluoride solution is drying on the teeth of one child, she goes on to the next, so that a minimum amount of time is lost.

I didn't quite get your second question. I thought it was concerned with the evidence that fluoride got into the enamel?

DR. MEDOF.—Yes.

DR. KNUTSON.—No, there is no specific evidence on that subject at this time. Dr. Armstrong and I—really Dr. Armstrong—tried to find out whether there were more fluoride in the enamel of topical fluoride-treated deciduous teeth extracted from the mouths of some of the children in the study groups than in the untreated teeth, and the results were negative. However, he used all of the enamel in making the chemical analyses, and it is not unlikely, and in fact, it seems logical and reasonable, that if the fluoride is taken into the enamel as we think it is, it is merely adsorbed on the very outer surface of the enamel, and therefore the relative amount of fluoride in terms of all the enamel is extremely small.

DR. EASLICK.—Dr. Fosdick, do you still have a question?

DR. L. S. FOSDICK (Northwestern University).—Yes. Dr. Knutson, I should like to know the logic whereby you arrived at placing the fluoride on the teeth and letting

it dry. Because if this is an ionic reaction, or an ionic exchange, it is rather difficult to obtain that exchange from two dry substances. So, I am just wondering the logic back of that.

DR. KNUTSON.—I am not sure that I understand your question. Maybe I should let Dr. Armstrong take care of it, but if I do understand it, when the surfaces are thoroughly wetted that should, it seems to me, permit the passing of the fluoride ions into the enamel. Now, the drying part of the procedure was a carry-over from the first studies where we were concerned about not getting fluoride solution on the control half of the mouth. In subsequent studies we changed only one of the variables at a time, but the drying part of the technic didn't seem to be one of the first that we should be concerned with changing in our efforts to determine what is the most effective fluoride solution, what is the best concentration, the minimum number of applications, and so forth. Does that answer your question?

DR. FOSDICK.—Yes.

DR. EASLICK.—Dr. Armstrong, did you care to comment?

DR. W. D. ARMSTRONG (University of Minnesota).—I have been a little disturbed this morning about the suggestion that one could drink six ounces of 2 per cent sodium fluoride solution. A dose of four grams of sodium fluoride marks the limit before the safe and the dangerous dose to a human being is derived from experiments in which people have been accidentally killed with sodium fluoride and a guess made as to how much sodium fluoride they got. For example, a cook used sodium fluoride instead of sodium chloride in making pancakes, and an attempt was made to guess how many pancakes those persons ate who died and how many those ate who survived. From these estimates as to how much sodium fluoride was consumed, the fatal dose figure was guessed. Now, on the other hand, we should not be too worried about this matter because I think it was shown that the amount of sodium fluoride solution of 2 per cent strength which is required to cover or to wet the entire tooth surfaces of a child under the conditions outlined by Dr. Knutson is one cubic centimeter. Even if the child swallowed all of this, he would certainly be within the safe limit. Most of it runs down off the tooth and is collected on the cotton rolls which are not ingested, but are discarded.

DR. EASLICK.—I am sorry. I think Dr. Kerr wanted the floor. We shall have one more question; then we have to take the next speaker.

DR. DONALD A. KERR (University of Michigan).—I think there is a little evidence from electrophotomicrographs that this process of fluorine in the teeth is a surface deposition rather than an absorption of fluorine into enamel surface, or into the enamel. Gerould has some pictures of electrophotomicrographs, in which the teeth have been treated with fluorine. The pictures show that the fluorine is a surface mechanism.

DR. EASLICK.—Dr. Gruebbel?

DR. ALLEN O. GRUEBBEL.—Thinking in terms of the use of this procedure by the practicing dentist — (and I am sure that Dr. Knutson hopes that the practicing dentists will use this procedure routinely in their offices), I am concerned about some evidence which I don't believe has been presented so far as I know, and which you may have. It may help us in determining how we can answer some questions that are very frequently asked of us by the dentists. Whether or not the practicing dentists will adopt this procedure will depend upon the results they obtain with it in their own offices. In my opinion, they will not take the time to make a statistical record of the total number of children who receive the treatment. They are going to judge it, by and large, by the results on individual cases.

Now, my question is this. What has been the evidence with respect to the distribution of the reduction in dental caries in the total population of children? Was it limited to a small proportion of the children, or was it evenly distributed among the children treated in relation to their caries susceptibility?

DR. KNUTSON.—It was distributed about as one would expect it to be distributed, that is, according to what we frequently refer to as a normal distribution. Most of the children showed a reduction; some children apparently had a greater reduction than others; and a few of them, none. But I can say that the reduction was rather uniformly distributed among the children. In other words, it was a common thing to see, if a child had caries, a cavity on the untreated side and none on the treated side, three on the untreated side, and two on the treated side, and rarely the reverse. It has been my impression among dentists who are using topical fluoride and who have used it for some time that they are convinced in their own practices that the results are quite evident.

DR. EASLICK.—Out of courtesy to the next speaker, I shall have to close the discussion here, and we shall have some more discussion of fluorine after the last speaker this morning; so keep your discussion and questions available.

Inasmuch as the topical application of silver salts does have some characteristics similar to the topical application of fluorides, Dr. Helmut A. Zander very graciously consented to help us out by discussing, "The Effectiveness of the Topical Application of Silver Salts in the Control of Caries."

Dr. Zander comes from Tufts College Dental School in Boston where he serves as Associate Professor of Clinical Dentistry. It is a real pleasure for me to present him to this audience. I doubt that anyone has taken his assignment more seriously and worked more conscientiously than Dr. Zander has on his particular assignment, and I am sure that you will be interested to hear what he has to present. Dr. Zander, you have the next thirty minutes.

THE EFFECTIVENESS OF THE TOPICAL APPLICATION OF SILVER SALTS IN THE CONTROL OF CARIES

HELMUT A. ZANDER, D.D.S., M.S., ASSOCIATE PROFESSOR OF CLINICAL DENTISTRY, TUFTS COLLEGE DENTAL SCHOOL, BOSTON, MASS.

There isn't anything new about the topical application of silver salts, or as it is now called, "impregnation." It was described in 1897 by Frank. Since that time, its use has been continued with reports of both success and failure to accomplish protection of teeth against dental caries. Quoting myself from a paper in 1941, "Too many times in the past, forceful personalities and wishful thinking have been the substitutes for carefully controlled experimentation. Nowhere in dentistry can this be better seen than in careful examination of the available literature on silver nitrate and its application in the treatment of dental caries." This statement still holds. Therefore, if your Committee, after I have made such a statement, still asks me to discuss this subject, I hope you won't be too surprised if I disregard the literature connected with the names of Brooks, Bryan, Cleary, Hoffman, and Prime, and a good many others. The reason for not considering them is found in the statement I read.

I should like to discuss the subject under two headings: 1. Does topical application of silver salts protect tooth substance against caries? 2. If it does, what is the mechanism of this protection?

Miller treated dentin and enamel of extracted teeth with a saturated silver nitrate solution. He then suspended the teeth in a glucose-saliva mixture and found that the area of dentin treated with silver nitrate did not show as much decalcification as did the untreated areas. This he could not duplicate with enamel. Miller's experiments were repeated by Hill and Arnold in 1936. They observed that enamel treated with silver nitrate did not decalcify as rapidly as untreated enamel when it was exposed to 5 per cent lactic acid. This is the laboratory evidence which I should like to present and discuss later.

Now, for clinical data. Hoffman subjected fifty-six case records of Prime's practice to statistical analysis. Prime had applied a solution of ammoniated silver nitrate to etched tooth surfaces; the interval between individual applications varied from three to more than six months. The number of etched areas treated were 887, and the total number of treatments, 5304, which gives about six treatments for each surface. According to Prime, "Caries was arrested in each case." Hoffman studied Prime's record cards and recalled those patients who had had silver nitrate applications at one time or another and examined those surfaces, which he found to be caries free. I hope Hoffman will discuss this study later. There is no control in this study. However, one can say that the author's figures indicate that, at least in the hands of Prime, silver nitrate impregnation was extremely successful in the prevention or control of dental caries.

After Dr. Wallace's excellent paper on the appraisal of dental literature and Dr. Knutson's objective report with such statistically significant results, it is easy to see that the available reports concerning silver nitrate and dental caries are worthless.

In complete contrast with Prime's work are the findings of Klein and Knutson in a well-controlled clinical study of 700 children 5 to 12 years of age. They used the same technic as Prime on the right maxillary first permanent molar and the left mandibular first molar. The other two first molars were used as controls. Once a year the teeth were re-treated, and they were re-examined after 3½ years.

Table I (from Klein and Knutson) shows in detail the condition of the teeth after three and one-half years. Let us look at the "caries-free" in 1937) column; by 1941, one or more surfaces became carious in 246 treated and 236 untreated maxillary first molars, and in 194 treated and 225 untreated mandibular first molars. On a percentage basis, 79 per cent of the treated and 77 per cent of the untreated molars became carious. Thus, the treated and untreated teeth suffered approximately the same caries incidence.

TABLE I

CARIOUS CONDITION, IN 1941, OF TREATED (T) AND CONTROL (C) FIRST PERMANENT MOLARS, SHOWING SPECIFIED CONDITIONS IN 1937, NUMBERS AND PERCENTAGES BASED ON DENTAL EXAMINATIONS OF 474 SCHOOL CHILDREN*

CONDITION IN 1937	NUMBER OF SURFACES ATTACKED BY CARIES IN 1941	FIRST PERMANENT MOLARS											
		NUMBER						PERCENTAGE†					
		MAX.		MAND.		BOTH		MAX.		MAND.		BOTH	
		T.	C.	T.	C.	T.	C.	T.	C.	T.	C.	T.	C.
Unerupted	One	42	32	19	21	61	53	54.5	42.1	29.2	31.8	43.0	37.3
	Two	13	7	17	15	30	22	16.9	9.2	26.2	22.7	21.1	15.5
	Three	0	1	5	4	5	5		1.3	7.7	6.1	3.5	3.5
	Four or five	1	0	8	7	9	7	1.3		12.3	10.6	6.3	5.0
	One or more	56	40	49	47	105	87	72.7	52.6	75.4	71.2	73.9	61.3
Caries-free	One	149	163	78	97	227	260	49.5	50.9	30.8	35.1	41.0	43.6
	Two	73	51	64	78	137	129	24.3	15.9	25.3	28.3	24.7	21.6
	Three	11	11	23	22	334	33	3.6	3.4	9.1	8.0	6.1	5.5
	Four or five	13	11	29	28	42	39	4.3	3.4	11.5	10.1	7.6	6.5
	One or more	246	236	194	225	440	461	81.7	73.8	76.7	81.5	79.4	77.3
Carious, one surface	Two	15	13	23	15	38	28	19.7	21.3	21.1	15.8	20.5	17.9
	Three	13	7	7	10	20	17	17.1	11.5	6.4	10.5	10.8	10.9
	Four or five	20	18	61	60	81	78	26.3	29.5	56.0	63.2	43.8	50.0
	Two or more	48	38	91	85	139	123	63.2	62.3	83.5	89.5	75.1	78.8
Carious, two surfaces‡	Three	1	3	1	1	2	4	6.3	20.0	3.1	4.3	4.2	10.5
	Four or five	8	8	28	18	36	26	50.0	53.3	87.5	78.3	75.0	68.4
	Three or more	9	11	29	19	38	30	56.3	73.3	90.6	82.6	79.2	78.9

*From Klein and Knutson: Studies on Dental Caries. XIII Effect of Ammoniacal Silver Nitrate on Caries in the First Permanent Molar, *J. A. D. A.* **29**: 1420, 1942.

†The number of teeth showing specified conditions in 1937, and on which the percentages are based, are shown in Table II (of Klein and Knutson).

‡Thirty-five molars, nineteen among the T group and sixteen among the C group, have been omitted from the analysis, since they showed carious lesions on three or more surfaces or were filled or extracted.

The latest proponent of the silver impregnation theory is Gottlieb. His clinical evidence is based upon the studies by Younger published in Gottlieb's book, *Dental Caries*. Twenty-nine children between the ages of 7 and 12, who had an average of 10.8 cavities per child, were selected for the experiment. All teeth with clinically visible carious lesions were then restored. Twenty-four children received an impregnation with 10 per cent silver nitrate solution precipitated by calcium chloride. Five children were used as a control group. At

the end of one year, the children were re-examined and at the same time re-impregnated. At the end of two years, nineteen children of the experimental group and five of the control group were re-examined. Now, ordinarily we could simply state that such a clinical study should not be discussed at all because obviously the number is insufficient. Because of the extreme interest in Gottlieb's work, and because of the publicity which it has received in the past few months, I have studied these figures in detail. I found out how many cavities Child No. 1 had, Child No. 2, and so on down the line; the age of each one of the children; and then, with the kind help of Miss Hall, we took these figures and tried our best to see whether there were some statistical significance. If you will just bear with me, I shall present some of those figures on the board, realizing of course, that the data actually do not merit discussion.

First Year of Experiment:

Control group	4.20	±	0.38 new cavities
Experimental group	0.46	±	0.23 new cavities
Difference	3.74		0.44

$$\text{Critical ratio} = \frac{3.74}{0.44} = 8+$$

Second Year of Experiment:

Control group	5.20	±	0.38 new cavities
Experimental group	0.68	±	0.21 new cavities
Difference	4.52		0.43

$$\text{Critical ratio} = \frac{4.52}{0.43} = 10+$$

These are the figures for the first and second years. In the first year, the incidence for the control group was 4.20 cavities plus or minus 0.38, while the treated group had 0.46 plus or minus 0.23, with a difference of 3.74 plus or minus 0.44; the critical ratio, 8 plus. I am not much of a statistician, but they tell me this is significant, if you are satisfied with those figures.

In the second year, the incidence of decay in the control group was 5.20 plus or minus 0.38; in the experimental group, 0.68 plus or minus 0.21; the difference, 4.152 plus or minus 0.43. The critical ratio equals 10 plus.

There were five subjects in the control group. In the first year those subjects had 4.2 cavities, and in the second year they had on top of that, 5.2 cavities. In other words, in two years, they got 9.4 cavities. The age group is 8 to 12 years. Those figures are way out of range of the figures which we know are the normal caries incidence for that age group. I don't think that having just five cases in the control group justifies statistical analysis. We do know something about the incidence of caries in such an age group, maybe not exactly for Dallas, Texas, where those figures come from, but for the nation as a whole. According to Klein, those figures are something like 1.4 cavities per year. To subject to rigid statistical analysis Younger's finding of 0.46 ± 0.23 new cavities in the silver nitrate-treated group, let us double the standard error and add this to the average, which gives us 0.92. This compared with Klein's national average is a little more in line, but still significant.

The problem is the interpretation of these studies by Younger, Klein, and Knutson, and also the data reported by Hoffman. On the surface, these studies give contradictory results which cannot be reconciled. Taking for granted that both studies were carried out in good faith, I shall try to explain why I believe

they may have arrived at their conclusions; Klein and Knutson, that there is no value to impregnation with silver nitrate; and Younger and Gottlieb, that it has value as a caries control measure. The age group is the same for both of these studies. One difference is that Klein and Knutson used only two first permanent molars for impregnation. Younger impregnated all teeth. Yesterday I asked the question of Dr. Arnold why there existed in endemic areas a difference in the reduced incidence of caries for some teeth when compared with others. A similar question was asked again today: which teeth and which surfaces apparently have a reduced incidence of dental caries? Robinson's answer applies also for the studies on silver nitrate. Klein and Knutson, I believe, devised too difficult a task for silver nitrate by expecting it to protect the first permanent molar. The incidence of caries is so much greater for this tooth than for any other tooth in the mouth that even in a relatively caries-immune mouth, this tooth often shows a carious lesion, although none of the other teeth have any evidence of decay. Silver nitrate impregnation may afford the tooth some resistance to caries, but not complete resistance. As far as the first molar is concerned, that increased resistance is not sufficient. To illustrate, let us assume that silver nitrated enamel is five times more resistant to decalcification than untreated enamel. Then it would take possibly five times as long for the carious process to get started, or the process has to be five times as strong. Now, these figures and this whole principle are just empirical for the sake of illustration. Klein and Knutson impregnated the first permanent molar once a year. The last impregnation was in the fall of 1939; the examination was made in the spring of 1941. A year and a half intervened between the last impregnation of silver nitrate. It is indeed possible that the caries attack rate for the first permanent molars may far exceed any artificial caries immunity. Perhaps an experiment in which sodium fluoride was applied to the permanent molars only would give comparable results. I am thinking now of the over-all 60 per cent reduction in dental caries due to sodium fluorides which is probably an average of 90 per cent effectiveness in the anteriors, and 30 per cent or less in molar teeth. Therefore, it seems logical that while for the first molar the silver nitrate impregnation was not sufficient protection, it may have been sufficient for some of the other teeth. The caries mechanism in all its complexity is more acute for the first molar than for any other tooth.

Younger certainly used much too small a group to stand up under critical examination. However, his work gives an indication that the impregnation method may have some value in reducing the incidence of caries. Much larger groups must be studied before Younger's and Gottlieb's claims can be accepted.

Now, a few words as to the mechanism by which the impregnation method might protect the tooth. Prime, Ross, and Gottlieb believe that it forms a barrier to the organic invasion roads of dental caries, and that brings us back to the mechanism of dental caries. In previous discussions at this meeting (according to Miller's hypothesis), it has been agreed that in caries of dentin a proteolytic action occurs, while for caries of enamel it is primarily an acid action. Gottlieb believes that the organisms which are capable of both proteolytic action and acid formation in the dentin are the same organisms respon-

sible for enamel caries. Gottlieb states, and I should like to quote from his book, "In case of invasion of a lamella, where the yellow pigmentation forms the spearhead, the possibility of any preparing action by acid is out of the question. Only when invading along the prism sheaths does acid form the spearhead. And here the situation merits discussion. Although exceptions may be found, acid alone rarely penetrates to the dentin through thick enamel without any trace of yellow pigmentation. It appears more probable that the proteolytic action in the already invaded enamel parts facilitates progressing penetration of additional acid producers. This is true only for enamel. In unhampered progressing dentin caries, the signs of acid action make the spearhead as a rule." In other words, Gottlieb, who uses yellow pigmentation and proteolysis interchangeably, believes that this is the first sign of caries which starts at a lamella. In other types of beginning caries, acid action is of prime importance. He feels that the difference is that the lamella organic invasion roads, as he constantly repeats, are the more common beginning areas of caries. Therefore, an obstruction of these roads by an insoluble salt is his explanation of the mechanism of silver impregnation. I should like to point out that he now recommends a 50 per cent solution of zinc chloride followed by a 10 per cent solution of zinc ferrocyanide instead of the silver impregnation we are talking about today. The principle of its action he claims to be the same as in the use of silver nitrate. Now, the action of silver nitrate solutions is not just one on the organic substance of the enamel. Arnold and Hill demonstrated, on powdered enamel, that the silver combined principally with the inorganic material of the enamel; only a small fraction of the silver salts were found in combination with the organic substance. While these experiments carried out on powdered enamel are not directly applicable to the intact enamel of human beings, they indicate that we do not just simply obstruct "the organic invasion roads," to use Gottlieb's terminology.

Therefore, in summary, the mechanism by which the application of silver salts on enamel affords protection may be an obstruction of the organic material or an alteration of the inorganic material to make it more resistant to decalcification by acids.

Now, before I conclude, I believe I have about two or three minutes more?

DR. EASLICK.—Yes, go ahead.

DR. ZANDER.—I should like to make a plea for silver nitrate. After you have heard about the fine studies on topical applications of sodium fluoride, this, as I mentioned, is an anticlimax. My job would be much more pleasant if I were able to show you a table of good data and a controlled experimental setup to prove that silver nitrate is worth something or is not worth something. Considering the available evidence, I, for one, cannot conclusively state that silver nitrate solution affords protection to the enamel of teeth. On the other hand, I would not completely discard the theoretical approach of Gottlieb, the thorough work of Klein and others, nor Hoffman's observations of Prime's work. What we need is a comprehencive study comparing the relative value of topical applications of silver nitrate and sodium fluoride. Once and for all, silver nitrate should have an opportunity either to prove or disprove itself. There are many public health workers

here; perhaps one of them will go home with the possibilities of silver nitrate in mind as well as the actualities of sodium fluoride.

Thank you.

DISCUSSION

DR. EASLICK.—Thanks very much, Dr. Zander. And I should like to assure you, for whatever the evidence is worth, my shirt never had dental caries.

Dr. Zander's presentation is now open for discussion.

DR. J. M. WISAN (New Jersey State Department of Health).—I should like to ask a question of both Dr. Zander and Dr. Knutson. In the light of their studies, do they consider that the filling of pits and fissures, which a sharp explorer detects, without observable decay is a justifiable procedure?

DR. ZANDER.—My answer to this question is not based upon scientific experiments. This is an opinion made after seeing hundreds of child patients, that one is not justified in letting pits and fissures go with just applications of drugs. I do believe that they should be filled. This is a good point which you brought out, and I am glad I have a chance to speak for one more second. Looking over this program on the "Control of Dental Caries," we see much about many drugs, but no mention of good operative dentistry and some of the procedures of careful diagnosis, which in many an office have controlled dental caries to such an extent that for many years the people haven't lost their teeth.

DR. EASLICK.—Dr. Knutson, do you want to get on the record, too, since this question was directed to both of you?

DR. JOHN W. KNUTSON (United States Public Health Service).—Even before topical fluorides came on the scene, I was of the opinion that if children, or individuals, got to the dentist at regular intervals, as determined by the dentist, one wouldn't have to worry about filling pits and fissures which might not become carious. Certainly if children did get to the dentist at regular intervals, there would be plenty of teeth to fill that were actually carious.

DR. EASLICK: Dr. Hoffman?

DR. OLIN E. HOFFMAN (Iowa State Department of Health).—I should like to say at the onset that Dr. Zander certainly gave a very fine presentation and was very fair with all the contestants. Dr. Zander did not, however, bring out the fact that the technics used by Dr. Prime and Dr. Knutson were different. He had the impression that the technic of the use of silver nitrate to arrest caries was the same. Now, as I understand Dr. Knutson's technic, he merely applied the silver nitrate to the portion of the tooth with cotton, isolating the quadrant with cotton rolls. Is that right, Dr. Knutson?

DR. KNUTSON.—Yes.

DR. HOFFMAN.—You did not use the rubber dam in the application, did you?

DR. KNUTSON.—That is right.

DR. HOFFMAN.—Now, that is one of the chief points of difference, Dr. Zander. It will correct the large difference in the conclusions that were reached in those two presentations. Dr. Prime first gave a prophylaxis, and he did just the thing that Dr. Zander has been encouraging, which all of us wish to encourage; that is, good operative dentistry. Dr. Prime filled all of those occusal surfaces. They were all filled; he did not depend on silver nitrate to take care of the occlusal surfaces. Dr. Atkins and I treated forty children, ranging in age from 4 to 17, over a five-year period of time. We treated both the proximal surfaces and the occlusal surfaces twice a year. We finished with twenty children; the proximal caries was arrested, but the occlusal caries was not. You can't stop occlusal caries with silver nitrate. You can slow it down, but you can't stop it. But Dr. Prime would give a prophylaxis and then under the rubber dam would apply silver nitrate to all of the surfaces, the whole crown portion under the rubber dam, and then he would take the

dental floss into interproximal surfaces. He would rub in the silver nitrate on the surface of the tooth so that the silver nitrate would penetrate the plaque, or destroy the plaque, and rub it on the proximal surface. That is the reason why he obtained such good results over many years that he used it in his practice, but he did not depend upon it to arrest occlusal caries.

DR. EASLICK.—Is there anything further, Dr. Knutson?

DR. KNUTSON.—I am glad that you pointed out the differences in technic, Dr. Hoffman, because up until the time that that was pointed out, I rather objected to the comparison. We had 500 cases in our study, whereas Younger had only nineteen treated and five control individuals in his study. Furthermore, it is clear that the five persons in his control group had an extremely high caries attack rate, four to five decayed teeth per person per year, and a high average is one per year. We know from this that he had an exceptional control group. In spite of that extremely high rate, the application of statistical methods to the data and the calculation of standard errors appear to have given additional meaning to those figures to Dr. Zander, which I don't think is warranted. In making comparisons of these data, it is important that we be familiar with the characteristics of dental caries in population groups. There is great variability in the number of carious teeth among individuals. The average incidence may be three-fourths to one decayed tooth per person per year for large groups, but in exceptional individuals, it may be five, six, or seven, so that if we have a control group consisting of five individuals who are obviously exceptional cases, we need to be very careful about our interpretations.

Another point that Dr. Zander brought out that I think one has to strain a little bit to follow or to agree with is that since our re-examinations were a year apart, sufficient time may have elapsed to give the teeth treated with silver nitrate time to become carious to the same extent as the control teeth. Any real difference in attack rates, as has been demonstrated in studies concerned with topical fluorides and fluoride waters, is reflected in the age specific prevalence rates.

DR. EASLICK.—Dr. Zander, do you wish to comment?

DR. ZANDER.—Dr. Robinson is going to, I think.

DR. HAMILTON B. G. ROBINSON (Ohio State University).—There is a very important point that is beginning to develop here, that there seem to be two groups of ideas on what dental caries control is. There is a group which thinks we are discussing dental caries control as a mass control measure only; and there is another group that has the concept "What is dental caries control for my individual patient?" Now, the type of dental caries control which Dr. Knutson is talking about is mass control of dental caries. That is the way he is trained; that is the way he thinks; that is the way the Public Health people want it. I, for my part, am on neither side; I want to see both approaches developed. I want to be able to tell a student in school how to take care of his patient individually and how to reduce mass caries at the same time. The figures come out differently because of different approaches. You, Dr. Knutson, want to find an approach that can be used on a mass of people and which will reduce the caries of that mass of people. When Dr. Knutson said there was a 40 per cent reduction in caries with the use of topically applied sodium fluoride, that does not mean that if you use his method on a child in your office the child is going to have 40 per cent less caries. It means that in a mass of the population there will be a 40 per cent reduction. In a certain individual in your office it might not produce any reduction at all; in another one, it might entirely stop caries. The private practitioner has an opportunity to do more things. Every time a careful operator like Prime applies silver nitrate in an office, he not only applies silver nitrate, but he treats the patient and he takes care of the operative work. There still is no substitute for metal and plastics for filling holes in teeth. You can't fill them with fluorine; you can't fill them with orange juice, nor with vitamin D. You have to fill them with gold, amalgam, cement, or plastic. We have to remember that point in considering these opposed views. The man who applies silver nitrate carefully and gives a prophylaxis for individually selected, carefully treated cases may, and

very probably will, show a reduction in caries in those office patients. But the other group is looking for a method that can be applied to a mass of the population and bring down the caries rate without having to give all this type of individual care. So, I think that we have to think of two types of caries control: That which the men who are in the offices are going to take back to use, which might be far different from caries control as we think of it for a mass population purpose.

DR. KNUTSON.—I am sorry I must disagree again this morning, this time with the point that Dr. Robinson was trying to make. I don't think we are talking about different things at all. If a procedure is effective for the individual, then it should be effective for numbers of individuals. If it is a partial control procedure, such as the use of penicillin in the treatment of osteomyelitis, it might be effective in only 80 per cent of the cases and, therefore, you can't assure a given patient who has the disease that you are going to cure his osteomyelitis. The use of topical fluorides is a partial preventive, and we can't predict which individual is going to get the greatest benefit from it. As I see it, there is no real difference between effective public health procedures and effective procedures used by the private practitioner. What difference there is, is based wholly on practical considerations. From a public health standpoint, procedures that don't cost too much and that can be applied effectively to the mass population are most likely to be adopted for use. I wonder if Dr. Hall might want to shed some light on this point of disagreement.

DR. EASLICK.—Dr. Hall, you have the floor.

DR. MARGUERITE F. HALL (University of Michigan).—Well, I think it is sort of wonderful to be a statistician on the outside and to be working with these professional people. I should like to point out that both Dr. Knutson and Dr. Robinson are right. It is wonderful to encourage the private practitioner to have a little research attitude and to try and analyze his data, even though the data are limited. And the value of some of the statistics, as Dr. Zander pointed out, is excellent to indicate that there is something in small samples when you have faith in the comparative value within the sample itself. And the professional man comes to the foreground in his consideration of that control group made up of the Texas people by saying "sure, those five cases are absolutely ones." It would be just as though in the height illustration we selected all the Alton, Illinois, giants. And that is what happened. But Dr. Zander tried to do something with the material by taking the large sample of control individuals, through some of the work that has been previously done, and still show that even though the control group on the large sample produced the 1.4, Dr. Younger from Dallas had an experimental group which still might be of some value. Now, there are other statistical interpretations that Dr. Zander didn't give which would come in if the material were really played out by the statistician, but usually the statistician drops from the scene as soon as the professional man has received the help that he has taken from the use of statistics as a tool. I definitely have been appreciating this work this week in seeing the recognition that the professional man is giving to all the tools that he can have at his disposal, and statistics happens to be one of those tools.

DR. EASLIK.—Now, Dr. Wisan, you requested the floor.

DR. J. M. WISAN.—I should like to suggest that we suspect the figure of one cavity per year in children. My basis for suspecting these data is the experience that we have had in New Jersey with approximately 10,000 children treated by approximately 100 different dentists who found an average of three fillings per year required per child.

DR. ZANDER.—I should like to get into this apparent dissertation between Dr. Robinson and Dr. Knutson. I actually let Dr. Robinson speak when I should have answered you in the first place, but I thought he could do it just as well, or probably better. Now, as I said, statistics are fine, but I also have some logic. I am a human being; I am a dentist and see certain things. If I go home from this conference, as it has been developed, my clinic patients ask "What is the latest? Is fluoride all right?" And I say, "In 40 per cent of the cases sodium fluoride does reduce caries," or I say, "Forty per cent of the caries inci-

dence can be reduced.'' Now, how it looks in the individual case is of some importance both from a public health point of view and also from the private practitioner's point of view. If you apply this to a very susceptible patient, don't worry at all about the causes of his susceptibility. Don't worry at all about the mechanism of caries in his or in any other case. All you do in such a case is apply topically sodium fluoride. I don't believe that in this particular individual you will find that he has 40 per cent less decay one year from now. If you would take the individual whose caries susceptibility, as indicated by past and present experience, lactobacillus count, and so on, are fairly low, that individual who is on the other side of the statistics and only gets one or two cavities per year, you may find in that particular individual an eradication of dental caries. The reason for this may be found in the mechanism by which sodium fluoride protects the tooth and/or the caries mechanism. While this factor may not be so important to the statistician who studies a large number of figures for the whole population, it is very important to the individual dentist and to the individual patient. Perhaps some day we shall be able to predict those cases in which sodium fluoride will and will not be effective. This point, up to now, hadn't been brought out too well, and that is what I believe Dr. Robinson and I are driving at.

DR. KNUTSON.—I just want to rise to point out that I finally arrived at the conclusion that I was wrong. I misinterpreted the meaning of Dr. Robinson's remarks. My misunderstanding was on the point of essential difference between the interest of the public health worker and the private practitioner in the patient. I am sorry I made that misinterpretation of your remarks.

DR. ROBINSON.—I was going to say that was my intent, and I also want to say that I recommend that the individual dentist use topically applied sodium fluoride, but he is not going to see the results the same way you do. You are seeing a 40 per cent reduction, and he might see a 100 per cent reduction or zero reduction. He has to think of the individual, and you have to think as a public health man.

DR. KNUTSON.—That is right. The point should be brought out, too, that by and large, dentists have not been accustomed to rendering preventive services. They don't know how to charge for preventive measures—charge their patients a fee for them. I agree with you and with Dr. Gruebbel, who brought out the point earlier that several problems remain to be solved in connection with this whole matter of using a preventive agent, and particularly, one which is a partial preventive.

DR. EASLIK.—Along about this time on previous mornings we had a ''wiggle'' period, and if some of you will keep your questions, although we are running far behind this morning, we shall have a five-minute wiggle period and then reconvene.

DR. EASLICK.—Ladies and gentlemen, if you will come to order, I should like to present the final speaker in this symposium on fluorine. He has arrived in Ann Arbor almost out of breath, because of a combination of problems which I understand arose from accepting a new position, from house hunting, and from the arrival of the stork, which I suppose one could classify as house breaking!

Anyhow, we certainly are glad to see you here, Dr. Bibby, and because of the unusual effort that you have exerted to join this Workshop group, I think you are almost entitled to the ovation that the Massachusetts hygienists have given you in a recent article.

Dr. Bibby comes very, very recently from Rochester, New York, where he has accepted the position of Director of the Eastman Dental Dispensary. He has, however, scarcely stepped out of the Dean's office of Tufts College Dental School. The subject assigned to him is ''Fluoride Mouthwashes, Fluoride Dentifrices, and Other Uses of Fluorides in the Control of Caries.'' That about covers the field, I think, Dr. Bibby, and I am glad to present you to this group.

FLUORIDE MOUTHWASHES, FLUORIDE DENTIFRICES, AND OTHER USES OF FLUORIDES IN CONTROL OF CARIES*

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I think probably the best way of introducing what I am going to report, a good deal of which is work which I have been instrumental in instigating, is to say that after the initial demonstration that there was a reduction in dental caries resulting from the application of sodium fluoride, it seemed to me that the important thing to do was to see if we could improve upon the effectiveness of the procedure which we had run upon purely in a hit or miss way. It happens that we had been associated for some time with investigations trying to determine the mode of action of fluorine in preventing dental caries. Out of those investigations we came to the tentative conclusion (perhaps we stated it more than tentatively) that fluorine was effective largely as the result of reducing the solubility of the enamel and thereby making it more resistant to an attack of dental caries. Obviously such an hypothesis has little value unless it has proof. Therefore, in starting the initial study on fluoride application, we had in mind two purposes. The first was to test the rightness of our theory on the mode of action. The second was that by chance we might strike something which would give rise to a new approach to caries control.

There were no guide posts; no one had ever before described a method for using fluorides in the mouth. Being cautious, we started off with one-tenth of one per cent solution of sodium fluoride. Since it was thirty miles to Brockton where we started our study, and since my car wasn't too reliable and the snows in New England are somewhat unpredictable, I arranged, as well as I could, to get down to Brockton in the better weather and at times convenient for the school system. That is why we gave treatments at intervals of approximately four months, or three times a year. The group of children was as large as we could conveniently collect. We started off with more than one hundred, and so had the mystic advantage of having crossed the century mark when we started. So this somewhat unscientific experiment was started and gave good results. From then on, my activities in the clinical fluorine studies have been rather unfortunate.

At this point we thought we would become scientific and make a laboratory approach to the problem and really find something which instead of reducing dental caries by 40 per cent as sodium fluoride does, might reduce it by 95 per cent, or even 96 per cent, and on up. Accordingly, we initiated laboratory studies with the idea of subsequently applying findings in clinical tests. That was one aspect of the problem. Another approach to improving the efficiency of fluoride therapy was based on the idea that since the somewhat casual method we employed in Brockton of bringing fluoride in contact with the tooth surfaces seemed to work, a modified clinical procedure might be much more effective. We envisioned a procedure which would not only be more effective but would also be

*A stenographic report of an oral presentation as corrected by the author.

simpler so that it could be more generally used both in dental practice and as a public health method. With those thoughts in mind, we carried out studies in both fields.

THE LABORATORY STUDIES

Our laboratory studies indicated that there were at least thirteen other elements which reduced enamel solubility a great deal more effectively than did fluorine. We thought that in those thirteen elements we might find something which would produce a greater reduction of dental caries. We also found that the solubility-reducing effect of fluoride was stepped up when we used an acid medium. The last finding was put to clinical application and clinical studies were alse instituted, some agents other than fluorides being used. So much for the laboratory background.

CLINICAL STUDIES

As to the types of methods with which we experimented to try to see if we could get a more generally applicable procedure, I can list the following: The use of lead fluoride, the use of acidulated fluorides, the use of indium nitrate, and some other agents.

The results of only some of these tests, about one-half of them, are now available, and here is the record of what we found, together with an analysis of work by others who have entered the field. We shall limit ourselves to considering uses other than topical applications of fluoride, and the use of substances other than sodium fluoride.

The Use of Mouth Washes.—Atkins first recommended the use of a sodium fluoride mouthwash. He used a mouthwash containing about four parts per million of fluorine applied after a thorough cleaning after meals. He reports results on twenty patients and found an 87 per cent reduction in the lactobacillus count in his group. We set up another study, using thirty-one students with a mouthwash which we concocted. At this time we had the idea of acidulated fluoride. This mixfure was made with a buffered sodium fluoride solution at pH 4, with a control mouthwash at pH 4 without the fluoride, and the students used it. It was pretty potent because I received complaints from the students that their landladies were complaining that it etched the sinks where they washed their mouths. The results in this group of thirty-one patients were negative when compared with twenty-nine controls. We got no result, no reduction of dental caries.

Not entirely discouraged by this result and feeling that the effect of fluoride might be more potent in youngsters than in mature students, we felt it necessary to set up a study with a younger age group. In this instance we had a more acceptable mouthwash compounded by the Upjohn Company, who supplied it to us. It was very pleasant; in fact, it tasted very much like orange juice, and we were a little bit frightened that children might want to drink it, it tasted so nice. The mouth washing was done under supervision. For a period of a year, 187 children used this mouthwash under supervision; we had a group of 157 controls who used an acidulated fluoride-free mouthwash. Now, everything was right. We had a young age group in which we knew we could demonstrate the reduction

of caries. We had a group which the statisticians can't sneer at too much. We had a mouthwash which was acceptable, and the mouth washing was done under supervision. The examinations were double checked by examiners who didn't know what groups they were examining. What did we find? After the use of this mouthwash for one year, there was not a decrease, but an increase of 32 per cent in the mouthwash group, a 32 per cent increase in DF teeth in the group using the fluoride mouthwash. Science is wonderful, the laboratory is wonderful, but it does you wrong sometimes, and it did us wrong this time.

It seems appropriate to comment on the unexpected results given by the acid mouthwash. We have no definite explanation; we have only a theory. We have been running animal groups, brushing the teeth with various fluoride mixtures. In several hamster groups in which the teeth were brushed with sodium fluoride, we obtained a definite caries reduction. But in a group in which we used the acidulated fluoride under the same circumstances, we had a doubling of dental caries. So, on the basis of the animal studies and the clinical findings, it would seem that the acidulated fluoride used as a mouthwash, or used as direct applications on the enamel, is definitely deleterious to the teeth. What happens? How do we account for it? I can't really account for it, but I do think that it is interesting to note that the studies which have been made on the mode of action of acidulated sodium fluoride on enamel (Rae and Clegg) have shown that a precipitation or a layering of calcium fluoride occurred on the tooth surfaces. Therefore, my tentative conclusion is that a layering of calcium fluoride on the tooth surface, which is presumably what resulted from our treatments with acidulated fluorides, isn't effective in reducing dental caries.

I lean a little bit toward the theory that perhaps when we decrease the pH of the solution, and temporarily the reacting surface, below the isoelectric point of the tooth we get adsorption phenomena; when the tooth surface reverts back to its normal pH the adsorbing processes are reversed, and perhaps the calcium fluoride layer just disappears. Perhaps when we use neutral fluoride solutions we get what other people have maintained, a change of the hydroxy-apatite of the tooth surface to a fluorapatite.

The Use of Dentifrices.—We have three studies here. In one, seventy-two youngsters 5 to 16 years of age using a liquid dentifrice containing fluoride, at the end of one year and of two years, despite a transient reduction of lactobacillus count, showed no reduction of dental caries, as compared with the control group. In two student groups, one using a paste containing fluoride and the other using a liquid dentifrice containing fluoride, we got no reduction of dental caries. This study, we think, should be discounted somewhat on the basis of the fact that this group was not of the age range in which the maximal caries reductions have been shown. The age factor may have been a contributing factor to these negative results.

The first group, which was composed of orphanage children, showed a peculiar thing. There was only one-third of the caries activity in both the control and the experimental group in the second year of the study (a war year) that there was in the first year of the study. There is obviously something

peculiar about this particular study which makes us a little suspicious of the results, and so we are not quite convinced that the fluoride won't work in a dentifrice.

I might say in passing that on the basis of Knutson's and my findings, a greater caries reduction in the upper teeth than in the lower teeth when one side of the mouth is treated, I have felt that perhaps the failure to get as big a difference between the treated and untreated side in the lower teeth is due to some of the fluoride passing into the solution or dissolving into the saliva and being carried to the control side of the lower teeth. To me, this indicates that it doesn't take much fluoride to give rise to a reduced caries activity. As a consequence, I can't really see why fluoride dentifrices won't work, but they do seem to work, and that is the state of the situation at the present time.

We also have a study by McClendon, as yet reported only in abstract. McClendon used a natural rock phosphate, which contains about 4 per cent fluorine and is presumably fluorapatite and also a synthetic fluorapatite, as a test of the effect of brushing the teeth. A student group of unspecified age was employed for a period of a year. As a result of using this powdered rock phosphate and synthetic phosphate, he claims a 60 per cent reduction of caries over a one-year period. He also claims to have produced a caries reduction in rats. In the rats the percentage of reduction isn't specified. It is a little hard for me to accept McClendon's findings without at least having his data. Apparently the fluorapatite was used as a dry powder. It seems very unlikely that fluorine in the fluorapatite is going to let go of that phosphate molecule and jump over to a similar one on the tooth surface. The fluoride is dry; it is not in ionized form, and it is very insoluble. I find it hard to see how the reported results came about, but they are in the literature and can't be ignored.

The Use of Prophylactic Cleaning Mixtures.—Another procedure which we followed was to incorporate fluorides in the cleaning mixtures used by dental hygienists. In Massachusetts, the hygienists are spreading the fluoride around in fine style. I didn't instigate it; the dentists wanted to do it, and I didn't try to stop it. It is an accepted practice there. Massachusetts, Maine, Rhode Island, and Connecticut are all doing it. I mention this for the interest of those who may face the problem in their states. We included the fluorides in a prophylactic pumice-hydrogen peroxide mixture. We used a 4 per cent sodium fluoride added in sufficient quantity to give a final concentration of fluoride in the cleaning mixture of 1 per cent. In school children, 6 to 15 years of age, one side of the mouth was cleaned, in one group of ninety-five, twice, and in one group of 47, three times during the school year, and examined after the period of a year. In those treated twice, we found a 25 per cent reduction of dental caries on the treated side. In the group treated three times, we found a reduction of 43 per cent. Admittedly the groups were small, but let me make a point in passing. My thought in my clinical studies has been that I have been playing for big game. If the percentage reduction was going to be so small that it wasn't pretty obvious in a group of 100 patients, then I wasn't very much interested. So I report these findings not as final statistical evaluations, or anything like that, but just as jabs in various directions to see if there were any procedure that would

give us more promising results than those we obtained by our original procedure of making topical applications.

Next, we expanded this group according to Knutson's specifications of 250 or more patients, set up a program of three cleanings per year, and turned it over to a dental supervisor and the hygienists. I went back and sat in my swivel chair and waited for the year to elapse—and waited three years for the results to be calculated from the punch cards. We have sampled the results by the old-fashioned pencil and paper method and found no reduction of dental caries. We used three prophylaxes, given by a dental hygienist without close supervision. What does that mean? It means either that it doesn't work, or that you have to be a little fussy about the technic that you use. The answer I do not know. We have other studies which will bring in results very shortly.

Lozenges and Pastels.—We have all heard something about the use of fluoride contained in lozenges. You know the calcium fluoride lozenges on the market which have been very skillfully promoted to appeal to the medical and dental profession, particularly the former. The work was done by Strean and Beaudet in Quebec. In talking to those close to this work, I find there is some question as to the significance of the work. I would question the results from several points of view. One is that the lozenges contain calcium fluoride. Like fluorapatite, calcium fluoride is not readily ionizable and probably wouldn't either react topically to any great extent or be adsorbed from the intestines as fully as other fluorides. I am also a little perplexed about Strean and Beaudet's figures because they report a 65 per cent caries increase over an eight-month period, which is 100 per cent caries increase per annum. A 100 per cent increase of caries per annum—that can't go on very long, because with his initial groups which he records as having three or more cavities. If this caries increase continued up to the age of 16, there would have to be something like 1,600 new cavities during that year. It is a little difficult to see how that could happen. In short, I am suspicious of the results.

Strean and Beaudet reported a 40 per cent reduction using calcium fluoride, vitamin D, ascorbic acid lozenges, one a day, sucked or chewed and swallowed. A very neat idea (I wish I had thought of it myself) for combining local and systemic usage. In another group using just the calcium fluoride lozenges, there was a 33 per cent reduction of dental caries. There was a control group of 220-odd in which there was the 65 per cent increase of caries I just spoke about. I can't feel that the calcium fluoride lozenge is in any way justified. I can say that at the present I know of a small experiment (I am not running it, I am only doing the lactobacillus counts) in which these lozenges are being tested again by the company. We shall not have the results of clinical tests until November. Three lactobacillus counts to date have not shown a significant reduction of whatever they show. We are independently running a group of studies using sodium fluoride lozenges without any vitamin content. We shall not have the results on that until I settle down to extract the data from the examination charts which I have with me. I am not optimistic about the result. In this study we are using coated sodium fluoride pills and a placebo tablet as controls.

Chewing Gum.—Another method which has been advocated for caries control is the use of a fluorine-containing chewing gum. It has been on sale in Massachusetts recently under the name of Flogum. I tried to discourage the gentleman who was putting this on the market, but he said how he had gone broke twice before and was prepared to go broke again. The Federal Foods and Drugs Administration gave him a license, and while there was a good initial sale, soon he had to show evidence that his claims were justified. This he couldn't do, and I don't believe this product is on the market any more. This fluorine chewing gum contained one part per million of fluorine. The promoter had been giving it to his children, and a number of dentists were distributing it. As usual, people had observed beneficial results but no proper clinical study was organized. As a matter of interest, I calculated how many pieces of this chewing gum would have to be chewed a day to give one milligram of fluorine to the child. It worked out something like over 400 tablets of chewing gum a day. It seems a little impractical to go very far on that basis.

Fluoride Bath.—A small group of about forty patients were treated with this method. It consists of making a fluoride bath from hydrocolloid or similar impression by hollowing out the area about the teeth. This is filled with a fluoride solution and placed back on the teeth for a period of twenty minutes or so. The results were essentially negative. The group was too small to follow further, and the results cannot be regarded as final.

Electrophoresis.—In the attempt to use the electrophoresis principle, we got a fairly satisfactory theoretical approach worked out. The idea bogs down on a practical point. That is that the potential necessary to force any significant amount of fluoride into the tooth surface is one which the patient can't bear comfortably because so far it hasn't been possible to get the tooth crown bathed in a fluoride solution satisfactorily without having a leakage of the electric current around the neck of the tooth. This causes irritation of the periodontal tissues. It may ultimately be possible to lick that difficulty, but so far we haven't been able to do it.

Other Chemicals Than Sodium Fluoride.—We have completed one study just like the Brockton study. It was set up exactly the same, with the same period between treatment, and using the same technic, using lead fluoride, so that we could get a comparison of the effectiveness of lead fluoride as compared with sodium fluoride. At the end of one year, we were unable to demonstrate any beneficial results from the use of the lead fluoride. In hamsters, brushing lead fluoride on the teeth, Dr. Lazansky of Tufts was able to demonstrate a reduction of caries comparable to that obtained by brushing sodium fluoride on the teeth.

At present, we have a study under way in which one quadrant of the teeth is being treated with lead and another with sodium fluoride, alternating between upper and lower teeth. On the basis of my own findings and Knutson's findings, my present feeling is that lead fluoride is another good laboratory dream that won't hold up. Why doesn't it work? As far as I have been able to find out from picking the brains of some of my chemical associates, the answer may be that some of the lead salts are soluble in ammonia compounds of one sort or

another, and thus reactions with lead may in some way predispose teeth to an attack to which they normally aren't subjected. There are, of course, a variety of possibilities of adsorption, and so forth, which may furnish better explanations.

The fluoride which I should like to try most, and on which I should like to have been able to announce results today, after this discussion of silver nitrate and sodium fluoride, is silver fluoride. There is no reason why it shouldn't work very well, but we have no results on that.

The study using other chemicals that in any way advanced it is one with indium nitrate. In this study, we have avoided using a fluoride to see if we could get a caries-reducing effect without fluorine. The indium salt animal studies have not given satisfactory results. The clinical study, on the basis of a preliminary sampling, does not seem to be giving a satisfactory result. That summarizes what I have to report.

I should like to make one or two comments. One is that it is rather nice to be able to get up like this before a group which is essentially made up of clinicians and be able to say, as one who is essentially occupied with laboratory work, that I fear I can exemplify by my own experience a very unhealthful situation. I refer to a tendency of the clinical investigators to sit around and wait for judgments from on high in the laboratories. Don't do it! The whole thing is common sense; common sense in all clinics is just as valuable as common sense in the laboratory. We can make high-flown theories, but there is no reason to believe that because we think they are good, they are going to be good. My only regret is that I ever published the data on acidulated fluorides and lead fluorides, and so forth. I suspect from a scientific point of view it should be published because it has some bearing on the mode of action of fluorine. But it is a little unfortunate, I think, to have the clinicians waiting for judgments from the laboratories because the laboratory conclusions are only as valuable as the common sense of the laboratory workers. I always felt, and I am on record several places as stating that what dental caries investigation needs more than anything at this time is good clinical observation. The dividends already paid in the fluorine field prove that.

The other point that I want to make is that I believe we have in fluoride therapy a more practical approach to mass reduction of dental caries than we have seen in the past. But let us bear in mind that it is only one of an infinite number of possibilities. If on the basis of the finding that fluoride therapy is good we abandon other methods of caries control, it would be just as silly as abandoning the aseptic precautions in hospitals because nowadays we have penicillin to combat the commonest of the infections which would result. If we were to do that in a hospital and depend upon a good therapeutic agent to clean up the mess we made by our neglect, we would increase the number of complications resulting from sepsis. Likewise, I fear that if everybody waits for fluorine to solve their caries problems we may also give rise to an increase in the amount of dental caries rather than a reduction.

Thank you.

DISCUSSION

DR. EASLICK.—I should like to thank Dr. Bibby very much for his presentation. And I would like to tell the group that we have some important business out here in front of the dental school very, very soon. We have perhaps ten minutes for discussion. Dr. Strusser, we cut you off last time; do you want the floor at this moment?

DR. HARRY STRUSSER (New York Bureau of Dentistry).—Well, I think Dr. Bibby covered the question that I wanted to ask, but I think it bears repetition at this point. We must be sure that the practitioner understands our statistical language. When we

speak of a 40 per cent reduction in incidence of dental caries and he sees 1.3 as the increment or incidence for the year, he says, "Well, suppose I do get a 40 per cent reduction, I will have a reduction of half a cavity, I still have to fill that cavity." That is his reasoning, and we have to be careful as to how these data are made available to the private practitioner so that he will understand it because, after all, we depend upon the private practitioner to do most of the work on the patients.

DR. WALLACE D. ARMSTRONG (University of Minnesota).—I believe it was Dr. Russell who yesterday told me that preparations of 2 per cent sodium fluoride upon standing would spontaneously reach rather high degrees of acidity. I wonder if he could tell us more about that?

DR. EASLICK.—Dr. Russell, apparently you have an opportunity of answering.

DR. ALBERT L. RUSSELL (State Board of Health, South Dakota).—An opportunity I am rather sorry to have received because I know very little about this. This is a question that is being asked me by a great number of men. And the work that has been done on it, as far as I know, is probably limited to a laboratory in Kansas City in connection, I believe, with Kansas City Western. In our own laboratory at home we have a solution which is eighteen months old. It has been stored in ordinary glass, and the pH has dropped to about 3.0. The question comes up as to whether that is a dangerous solution to use in the mouth. That was the question I asked Dr. Armstrong. It is a very strange thing. I have little exact knowledge about it. I just wondered if there might be some danger in using a stale fluoride solution.

DR. ARMSTRONG.—I am simply unable to understand how that result would come about unless some one came along and poured concentrated acid into the bottle.

DR. EASLICK.—Dr. Wallace, do you wish to comment?

DR. DONALD A. WALLACE (American Dental Association).—I agree with Dr. Armstrong that it isn't imaginable how that reduction in pH would occur. Some months ago in the Bureau of Chemistry, Dr. William Doty made up several solutions of sodium fluoride, buffered to different pH values, as low as pH 4.0. They were bottled in Pyrex, and after two or three hours at room temperature, a copious white precipitate formed which we supposed was silica or some silicous material. The solution of the distilled water was perfectly clear after, oh, I suppose, three or four months of standing. We haven't checked the pH of the distilled water solution so far as I know, the solution made in distilled water, but from a lower grade of sodium fluoride than the reagent quality, but considerable sediment that suggests iron and ammonium hydroxide was formed.

DR. THOMAS J. HILL (Western Reserve University).—May I ask Dr. Bibby if he would like to comment on the work of Shaner and Smith on the reduction of acidophilus counts by using a fluorine dentifrice, or did he omit this intentionally?

DR. BIBBY.—No, I didn't omit it intentionally. I know the report; I read it some time ago. I wouldn't say I know it well. But I couldn't quite make out what it was trying to prove—perhaps that was your reaction, too, was it?

While I am on my feet I might respond to a request. I was asked to mention the bone flour studies. Reduction of dental caries by bone flour doesn't come in the area of topical use except in so far as chewing bone flour pills might bring the fluorine of bone flour in contact with the tooth. The only clinical data I know of are from the study of Harootian which was carried out in Massachusetts. He had nine patients of uncertain age, mainly

adults I believe, some of whom had only nine teeth. At the end of a year he got no increase in caries. On the basis of that finding, you have at least two commercial products on the market as caries preventives.

DR. EASLICK.—Thanks, Dr. Bibby. Is there any further discussion this morning?

DR. HAMILTON B. G. ROBINSON (Ohio State University).—I should like to say one thing for the record. I enjoyed Dr. Bibby's paper.

Friday, September 12

Morning Session

(Auditorium, School of Public Health)

Dr. Easlick.—Ladies and gentlemen: I should like to announce that it is a continued pleasure to find you still here and so prompt and alert. It is really a source of great regret to me, however, that this is the last day on which I can introduce our important and distinguished faculty to you. But one aspect of Friday morning's program does give me a great bit of cheer: all of the feminine portion of this important faculty will be introduced today in one attractive package.

As you consulted your programs you probably noted that the morning schedule calls for an evaluation of diet or diets as caries control measures and that three speakers will present this evaluation. The first speaker comes from Minnesota. Dr. Armstrong recently sustained some surgery and comes here still a convalescent. I know that from his discussions which you have been hearing in the auditorium you anticipated a fine presentation. Without further words I shall introduce Dr. Wallace D. Armstrong, Professor of Physiological Chemistry at the University of Minnesota. I want him to know that we appreciate his getting up from a hospital bed, cutting short his period of convalescence, to attend this Workshop, and I think that I can speak for the rest of the Workshop group, Dr. Armstrong, when I say that we are all most appreciative. Dr. Armstrong's subject is "An Evaluation of the Role of Vitamins and Minerals in the Control of Caries."

AN EVALUATION OF THE ROLE OF VITAMINS AND MINERALS IN THE CONTROL OF CARIES

WALLACE D. ARMSTRONG, PH.D., M.D., PROFESSOR PHYSIOLOGICAL CHEMISTRY, SCHOOL OF MEDICINE, UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINN.

It has been possible to produce clear evidence that certain vitamins and minerals are required for the formation of teeth of normal structure. It has been very difficult, however, to demonstrate that mature teeth require these nutrients in amounts greater than are needed to prevent gross and obvious signs of deficiency in other tissues. My first task will be to review the evidence in support of these statements. I shall, thereby, indicate why it appears that a mineral or vitamin deficiency must be present during the period of tooth formation if the tooth is to be affected in a manner which could lower its susceptibility to decay. As a sort of after-piece to the first topic, I shall attempt to present a summary of the somewhat controversial evidence which has been produced to show that single mineral or vitamin deficiencies are important causal factors in dental caries.

The effects of inadequate diets on developing teeth cannot always be stated in quantitative terms. Our best device, at present, for indicating the importance of the effects produced by nutritional deficiencies on teeth is to compare these effects with those produced on another tissue, such as bone. When such comparisons are made, we shall see that in many circumstances, developing teeth appear to be less liable to alteration by metabolic abnormalities than is forming bone. We shall also see that mature teeth, in so far as any objective criteria are concerned, appear to be almost, if not entirely, removed from the direct influence of nutrition.

EFFECTS OF VITAMIN DEFICIENCIES ON TEETH

Severe deficiencies of vitamin A produce in developing teeth characteristic abnormalities of growth and structure. Teeth of continuous growth such as the incisor of the rat have furnished a convenient means of recording these effects. Wolbach and Howe[1] showed that the primary dental disturbance in vitamin A deficiency is in the developing enamel. This effect is an example of the specific requirements for this vitamin by epithelial tissues and by those tissues derived from the ectoderm. The photographs in Fig. 1 are taken from a recent paper by Paul and Paul.[2] Rat *0* received an adequate intake of vitamin A. The other animals received suboptimal amounts of this vitamin over a long period. Animals *1* and *2* exhibited a loss of the normal orange pigment and a black mottling of the incisors. Animals *3* and *4* showed a chalky white appearance of the incisor teeth.

While an early effect of vitamin A deficiency of the teeth is a result of failure of ameloblastic activity, which may result in enamel agenesis or hypoplasia, a prominent end result is seen in a disorganized structure of the dentin. Fig. 2, taken from the work of Schour, Hoffman, and Smith[3] contrasts in a diagrammatic way the structure of the dentin seen in traverse sections of the incisor of

a normally fed rat with that of an animal supplied an insufficient amount of vitamin A. Vitamin A deficiency resulted in an acceleration of apposition of the enamel-covered dentin, while that of cementum-covered dentin was markedly

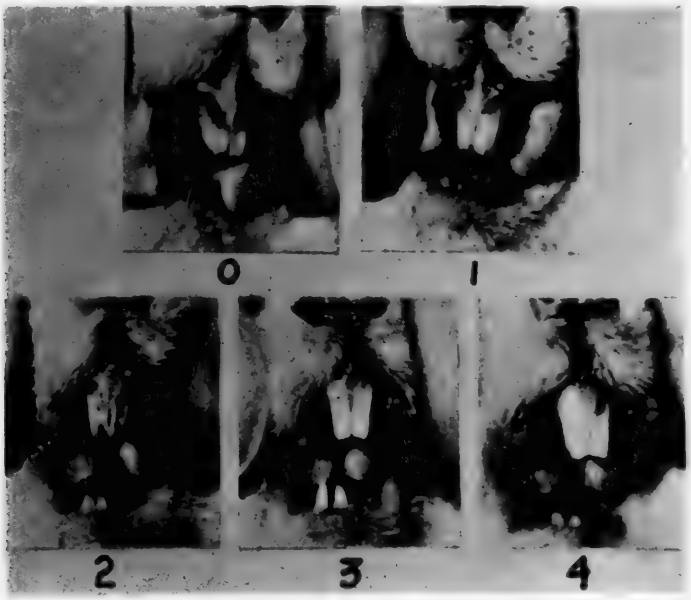

Fig. 1.—Effect of vitamin A deficiency on enamel of incisor teeth of the rat (Paul and Paul[2]).

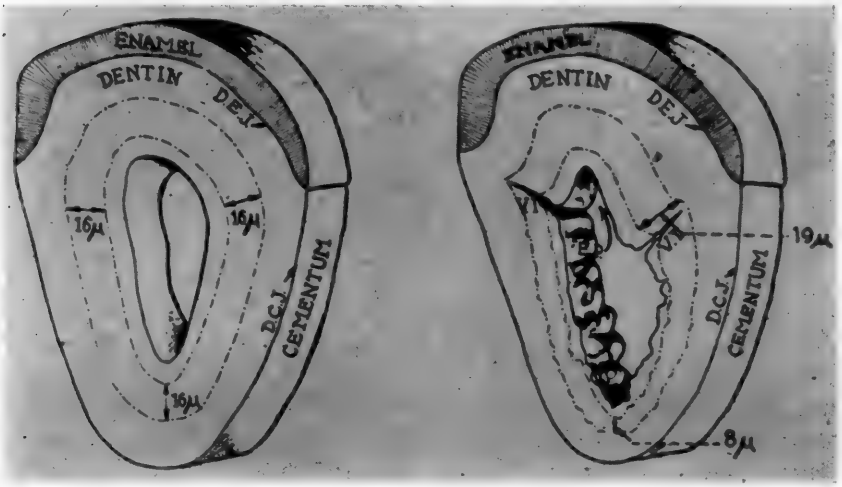

Fig. 2.—Effect of vitamin A deficiency on dentin of the incisor teeth of the rat (Schour, Hoffman, and Smith[3]).

decreased. This observation would suggest that the roots of teeth of limited growth, such as rat molar teeth, might be expected to show pronounced effects of vitamin A deficiency. However, because of the fact that rat molar teeth are very nearly completely formed before the vitamin A stores of weaned rats can be depleted, only minor abnormalities of molar teeth have been observed in the usual experiments. In order to produce earlier and more pronounced vitamin A deficiencies, Helen Mellanby[4] studied rat pups born of mothers fed diets deficient in this vitamin for twelve to twenty-five weeks. The roots of the molar teeth of the offspring subjected to the severest possible deficiency were misshapen, but these teeth were always less profoundly affected than the incisors.

While the developing tooth may be deleteriously affected by severe deficiencies of vitamin A, these structures appear to be considerably more resistant to the lack of this vitamin than most, if not all, other tissues. Many of the dental defects were produced only by diets so deficient as to exhaust the bodily store of vitamin A and, with continued feeding, to be incompatible with life.

The relation of the antirachitic vitamin to the formation of teeth of normal structure has been repeatedly demonstrated since M. Mellanby first noted, before vitamins A and D were recognized as separate entities, deformities in the teeth of puppies raised on diets deficient in the fat soluble vitamins. In Fig. 3, *a* and *e* are, respectively, the normal molar teeth of a child and of a dog.[5] On the right is shown increasing degrees of enamel hypoplasia of molar teeth of dogs produced by increasingly severe deficiencies of vitamin D intake. The hypoplastic enamel of the children's teeth is by analogy assumed to be also the result of variations in vitamin D available at the time of the development of the teeth. Lady Mellanby has emphasized these abnormalities of the surface of enamel of human teeth because she has championed the notion that hypoplastic enamel possesses an increased susceptibility to caries. This theory will be referred to in greater detail later. For the present, I should like to state that this relationship of vitamin D deficiency to dental caries is circumstantial since it is based on two independent and possibly unrelated observations. The first is the clear demonstration of the production of enamel hypoplasia by rachitogenic diets in certain experimental animals. Such hypoplastic teeth in lower animals do not, however, develop caries. The second observation is the apparent relationship of irregularities of enamel surface texture of human teeth to susceptibility to decay. At this point it may be mentioned that some doubt has been cast of the belief that enamel hypoplasia results solely from a vitamin D deficiency, since Weinmann and Schour[6] failed to produce this result in rats fed rachitogenic diets.

While the enamel disturbances are a prominent feature of vitamin D deficiency, it has been possible to study more satisfactorily the details of the production of the lesions in the case of the dentin. In the dentin as in the enamel, certain abnormal cellular alterations are produced, and there is also a marked failure of the growing dentin to calcify. This fact is well shown in several of the publications of Irving.[7]

The photomicrograph on the left of Fig. 4 is that of a longitudinal section of a rat incisor tooth after the animal has been fed for thirty-one days on the

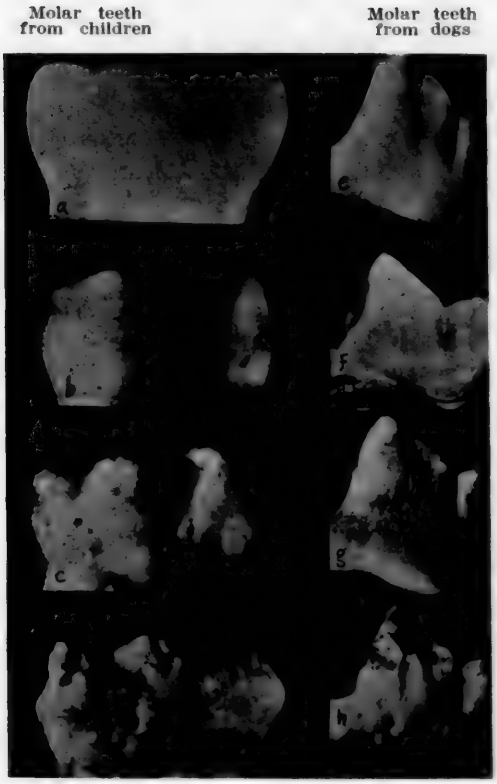

Fig. 3.—Effect of vitamin D deficiency on enamel of teeth of the dog and the child (M. Mellanby[5]).

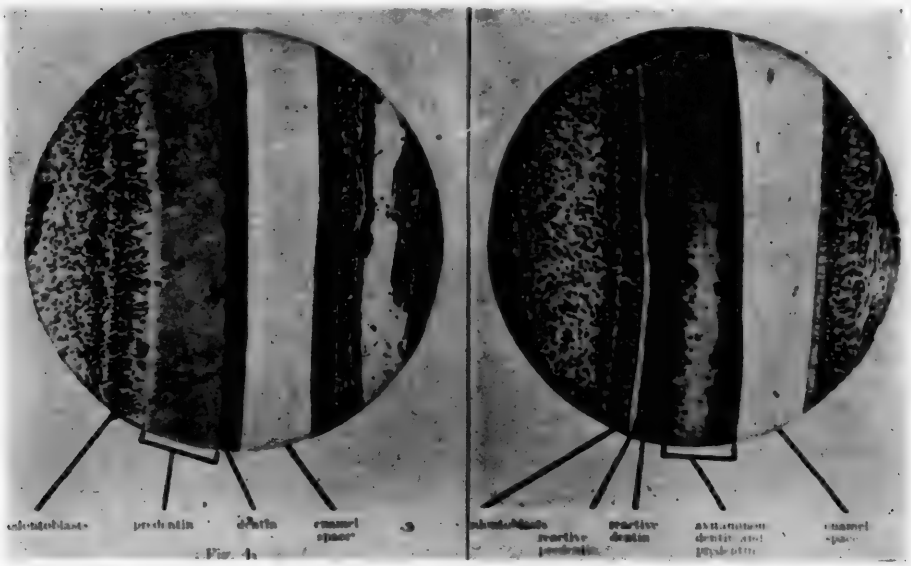

Fig. 4.—Effect of Vitamin D deficiency on dentin of the incisor of the rat and the effects of a single curative dose of vitamin D on the incisor dentin (Irving[7]).

rachitogenic diet used in vitamin D assay work. The predentin is dentin organic matrix which has not yet been calcified. Normally the predentin is quite narrow and is about the width shown for this structure in the section on the right of Fig. 4. This overgrowth of uncalcified dentin in the vitamin D-deficient animal has an obvious analogy to the wide epiphyseal cartilage of rachitic animals. The section on the right illustrates the effects produced by a single curative dose of vitamin D given ten days before sacrifice of the animal. After a delay of four to five days, dentin calcification began anew in quite a normal fashion in the predentin laid down after the vitamin was administered. By contrast, however, the calcification of predentin formed during the period of vitamin deficiency remained essentially unimproved. Irving,[7] in the same paper from which these illustrations were obtained, as indicated that the recovery of calcification by dentin is more sensitive to the effect of vitamin D than the healing of rachitic epiphyseal cartilage as measured by the line test.

The third vitamin required for normal teeth formation is vitamin C. The most prominent dental alterations are produced in the dentin with only minor, and possibly secondary, effects in the enamel. Depending upon the degree and duration of the avitaminosis, the formation of dentin is deficient and of disorganized structure. The classical work of Howe,[8] Westin,[9] and Hojer and Westin,[10] who showed that the fundamental pathosis in scurvy is a result of the failure of formation of the intercellular substance in tissues of mesenchymal origin, furnishes an explanation of the selective effect of vitamin C deficiency upon the dentin.

The requirement of vitamin C for normal dentin formation is higher than that of many other tissues, including bone, and for this reason dentin abnormalities may be produced in the absence of clinical signs of scurvy. As is well known, the gums and other investing tissues of the teeth of mature animals may be adversely affected in susceptible animals by a deficient supply of vitamin C. However, like vitamins A and D, there is no clear indication that ascorbic acid is required for the preservation of the integrity of the teeth themselves.

EFFECTS OF MINERAL DEFICIENCIES ON TEETH

Diets grossly inadequate in mineral elements may produce, in growing teeth, a deficient calcification, as well as changes in form and structure of the enamel and dentin. Nevertheless, considerable evidence has accumulated that teeth do not always undergo changes in ash or calcium and phosphorus content under dietary regimens which produce marked changes in growing bone.

Gaunt and Irving,[11] who studied the effects of diets of varying calcium to phosphorus ratio on bone and dentin, furnished one of the best indications of a fundamental difference in the metabolism of teeth and the skeleton. Fig. 5 shows the essential data obtained by Gaunt and Irving when young rats were fed a diet of high calcium to phosphorus ratio, namely four, and at several levels of calcium and phosphorus intake. At the lowest level of calcium and phosphorus intake the incisor dentin ash weight, as shown by the open bar, was essentially normal, but the degree of mineralization of the bones was very greatly

reduced. At higher levels of calcium and phosphorus intake the condition of the skeleton improved, but even at the highest levels the bones were entirely normal. Investigations of the same type were also carried out with diets of very low calcium to phosphorus ratio, namely, 0.5. In this case, the degree of mineralization of the dentin was definitely interfered with at the lower levels of calcium and phosphorus ratio. Thus, under conditions of inadequate calcium and phosphorus intake, it appears that the calcification of dentin and bone is facilitated by opposite conditions of dietary calcium to phosphorus ratio. Gaunt and Irving, therefore, suggest that in some fundamental way there is a marked difference in the factors governing the mineral metabolism of bone and teeth.

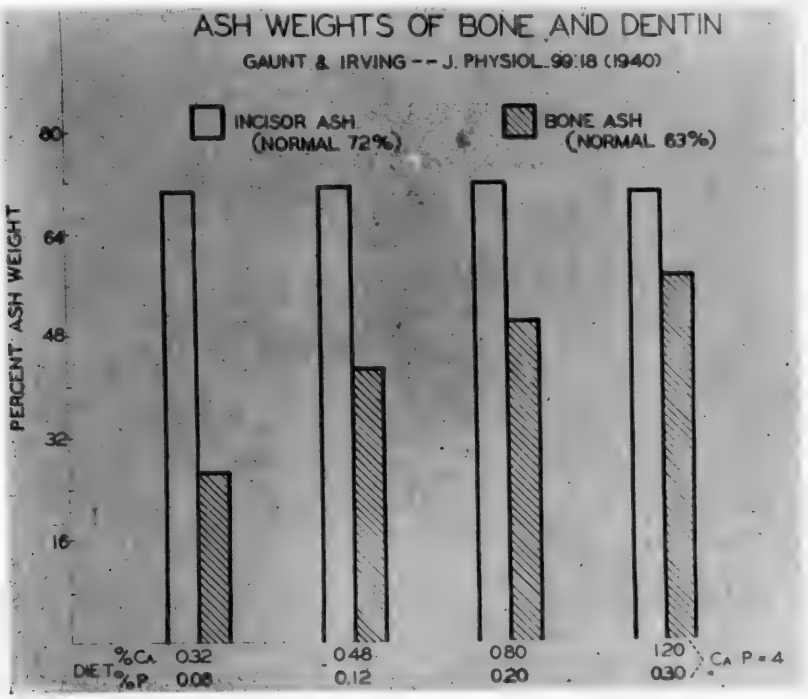

Fig. 5.—Effects of different levels of calcium and phosphorus intake at constant calcium to phosphorus ratio on incisor dentin and bone in the rat (Gaunt and Irving[11]).

Evidence from a number of directions indicates that teeth once formed are in comparison to the skeleton, essentially outside of the pale of nutrition. Albright, Aub, and Bauer,[12] and also Thoma[13] demonstrated that the teeth in cases of hyperparathyroidism were not affected even though the bones were severely decalcified. Schour, Tweedy, and McJunkin[14] were unable to find any evidence of calcium withdrawal from the molar teeth of rats subjected to parathyroidectomy and to repeated pregnancies. The same conclusion was reached from work of a different nature carried out in my laboratory.[15] Fully mature rats were fed, for 220 days, a diet severely deficient in calcium and vitamin D. During

this entire period the animals receiving the deficient diet were in negative calcium balance, but control animals fed an adequate diet were almost continually in the state of slight positive calcium balance. Certain of the averaged data obtained with the ten experimental animals are shown by the cross-hatched bars, and the correspondingly averaged data referring to the control animals, by the open bars (Fig. 6). The humeri of the animals receiving the deficient diet suffered some decalcification as shown by the relative heights of the bars

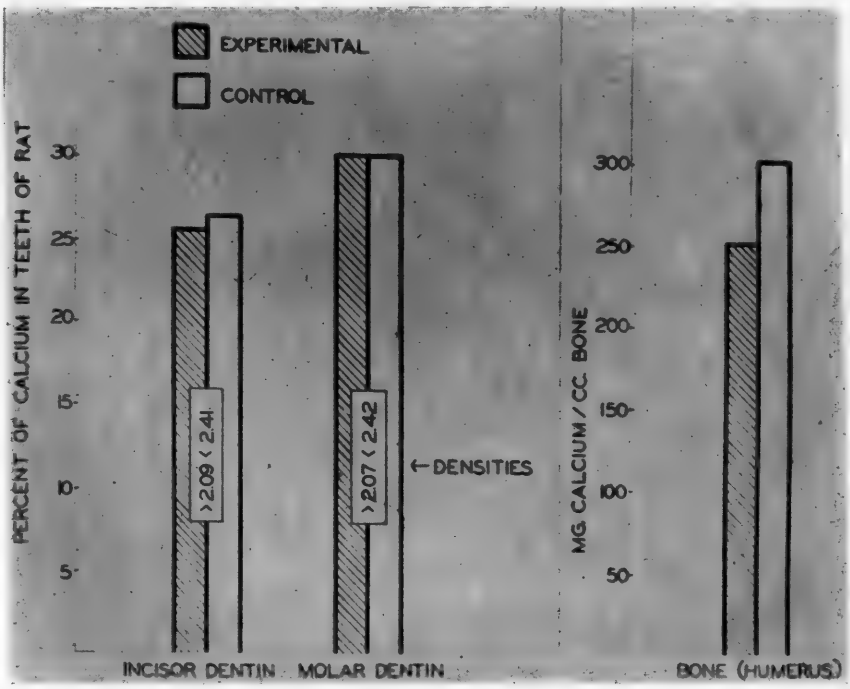

Fig. 6.—Effects of prolonged calcium and vitamin D deprivation on the dentin and on bone in the rat.

which indicate the mean calcium content of these bones in terms of milligrams of calcium per cubic centimeter of bone. The molar teeth of the experimental animals were loose in their sockets and the alveolar bone of these animals was thinned. However, these molar teeth appeared not to be affected with regard to calcium content. The molar teeth were separated into various fractions by the specific gravity flotation method for the isolation of enamel and dentin. For the purposes of the present discussion, only the data with reference to the dentin need be considered, although the enamel was also analyzed. Obviously there was no difference in the calcium content of the dentin derived from the molar teeth of the animals fed the inadequate and normal diets. In the case of the incisor teeth, which unlike the molars had been completely reformed several

times during the experimental period, there was a slight, but probably insignificant, lower calcium content in the dentin of the experimental animals as compared with that of the controls.

The radioactive isotopes have found considerable use in the study of the metabolism of mature teeth. Aub as early as 1938 demonstrated an uptake, under in vivo conditions, of radium D by dentin but not by enamel. When strong preparations of radioactive phosphorus became available, this isotope was applied to the study of mineral exchanges by enamel and dentin. Hevesy and I found[16] late in 1938 a small but measurable fraction of the total dose of labeled phosphate in the enamel of mature rats following the parenteral administration of potent doses of radioactive sodium phosphate. This uptake of labeled phosphorus by the enamel of mature teeth has been repeatedly demonstrated with a variety of animals and under different circumstances by work done in our own laboratory at Minnesota, by the University of Rochester group, and by the University of Chicago workers. The results have, in general, agreed in showing that the uptake of phosphorus by enamel is considerably less than that of the skeleton. Practically speaking, there are only two points now under discussion in this regard. First, the question as to the route by which enamel and dentin can acquire labeled phosphate, and second, the significance of the observation.

Campbell and Greenberg[17] first employed a radioactive isotope of calcium in a study of the exchange of calcium by various calcified tissues. Their data indicated that the uptake of radioactive calcium by the pooled incisor and molar teeth of the rat was as high as that of the skeleton when the data were calculated in terms of the dry weight of the tissues. Barnum and I[18] wished to study separately the exchange by the constantly growing incisors and by the molars, the last teeth in mature rats being constituted of fully formed enamel and dentin. Furthermore, it seemed of interest to study the enamel and dentin of these two groups of teeth separately. Greenberg very kindly supplied us with a liberal amount of radioactive calcium (Ca^{45}) for this experiment. Since the calcium and the phosphorus contents of the several calcified tissues are different, no direct comparison of the uptake of the radioactive element can be made between tissues when the data are calculated on the dry weight basis. It is necessary, in order to take into account the differences in composition of tissues, to utilize the concept of the specific activity whereby the uptake of the radioactive element is reported in terms of equal units of the element present in the several tissues. In Fig. 7 we have plotted molar specific activities which refer to the fraction of the retained dose found per mole of calcium or phosphorus. In this experiment the radioactive calcium and phosphorus were administered simultaneously to a single mature rat and the animal was killed five days later. Thus, it became possible to compare the relative rates of exchange of phosphorus and of calcium under the same physiological conditions. The relative rates of exchange for both phosphorus and calcium fall off in this order: femur marrow, femur epiphysis, femur diaphysis, incisor dentin, incisor enamel, molar dentin, and finally, molar enamel.

The fact that small amounts of radioactive elements can, under in vivo conditions, make their way into the dentin and enamel of fully formed teeth cannot be interpreted to indicate that the composition of these structure can be altered after the tooth is fully formed. It is quite possible that the radioelements which appear in enamel and dentin merely exchange or substitute for the normal isotopes of elements already present in these tissues. All that is demonstrated is the existence of a mechanism for transport of ions between dental structures and the circulation. In any event, this process in the case of the dental structures is feeble in comparison with that of the skeleton.

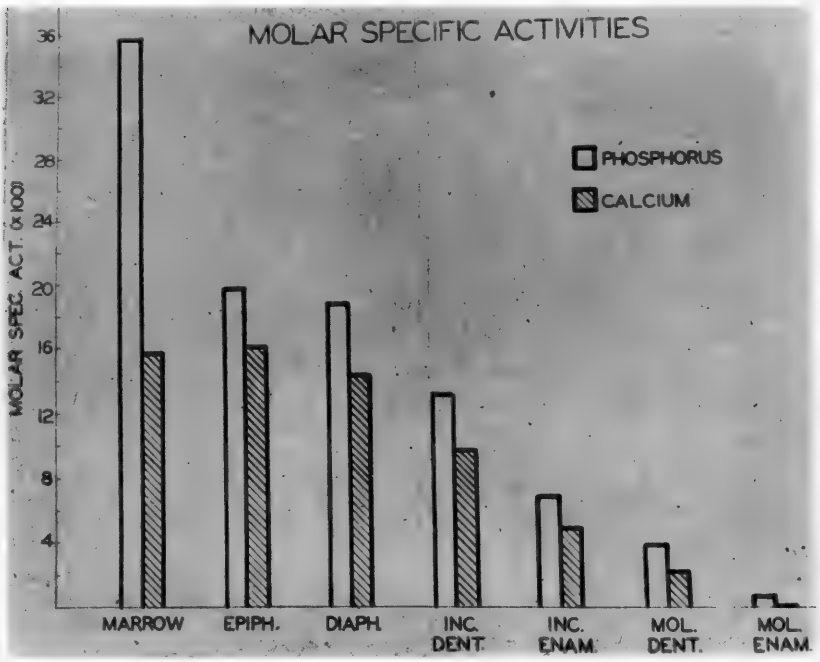

Fig. 7.—Comparative uptake and retention of radiocalcium and phosphorus by calcified tissues of the rat.

With the physiological background which I have tried to prepare in mind, I should now like to examine the evidence *pro* and *con* that single vitamin or mineral deficiencies can lower the resistance to decay of the teeth of the human being.

As a natural sequel to the demonstrated influence of vitamin D lack on tooth structure, a very great deal of emphasis has been laid on the possible role of dietary supplements of this vitamin as a means of reducing the caries attack rate and in slowing the progress of established lesions. These studies were first initiated and vigorously prosecuted in England by a group of workers under the direction and guidance of M. Mellanby.

As I previously indicated, M. Mellanby's notion of the mechanism by which vitamin D deficiency increases the susceptibility of teeth to decay is based upon

certain separate and distinct observations and on inferences drawn from these observations. These observations and deductions are: (1) Enamel hypoplasia can be produced in a variety of animals as a result of a deficiency of the antirachitic vitamin during the period of tooth formation. It is, therefore, assumed that hypoplasia of the enamel of human teeth is also a result of vitamin D deficiency. (2) Hypoplastic human teeth, all other conditions being equal, are significantly more prone to caries than are normally formed teeth.

At this point it is well to emphasize that the hypoplasia referred to by the Mellanby group and called M-hypoplasia is very different from the structural abnormality usually connoted by the word hypoplasia. The Mellanby group refers to a much less severe but more common condition than is detectable by simple visual inspection of the teeth. The condition of M-hypoplasia denotes certain microscopic changes in the structure of the enamel and is best discovered in ground sections of the teeth. However, it can be detected and graded in teeth in situ with the use of a fine tipped probe and a hand lens because of the subtle roughening of the tooth surface. By this means teeth can be graded into four groups, one normal and three degrees of hypoplasia.

Reference will later be made to some objections to each of the premises of the Mellanby school. I should now, however, like to indicate briefly the evidence derived from studies with the human being which M. Mellanby has brought forward in support of her thesis. It will be recalled that while in certain species of experimental animals, enamel hypoplasia can be produced by feeding diets deficient in vitamin D, these hypoplastic teeth of animals nevertheless do not become carious. M. Mellanby suggests that the failure of such hypoplastic teeth to decay is a result of the operation of some unknown protective factor in lower animals. She and her co-workers have described in several publications[19] their extensive observations that M-hypoplastic teeth of the human being are more prone to caries than normal teeth. Thus, accepting her thesis that hypoplasia of human teeth is due to vitamin D deficiency during the period of enamel formation and accepting her observations as to the increased caries susceptibility of such teeth, we have strong circumstantial evidence of the relation of an adequate vitamin D intake during infancy and early childhood to caries resistance.

A number of workers, notably Bibby[20] as well as Anderson, Williams, Halderson, Summerfeldt, and Agnew[21] have confirmed the Mellanby group with regard to the susceptibility of M-hypoplastic teeth to decay. However, Taylor and Day[22] and Day[23] alone have challenged the notion of the lessened resistance of hypoplastic teeth to caries. These workers have published several studies dealing with observations of rachitic children in India. They found no correlation between the incidence or extent of the bony stigmata of rickets and enamel hypoplasia.

If the thesis of the Mellanby school is correct, we would have an indication that a fair proportion of children receive suboptimal amounts of vitamin D in their regular dietaries and as medication. In this connection it is worthy of mention that vitamin D deficiency, as evidenced by rachitic changes in the bones discoverable only by histologic examination, is probably more common

than would be supposed from the incidence of clinical rickets. Follis, Jackson, Eliot, and Park[24] examined stained sections of the ribs of 230 children who died between the ages of 2 and 14 years. Evidence of rickets was found in 46.5 per cent of all the cases. The greater incidence of this histologic type of rickets occurred in the younger age groups and in those dying of acute illness. In only five of the cases was the bony condition detectable by roentgenograms of the skeleton during life. If this condition of occurrence of subclinical rickets, described by Follis, Jackson, Eliot, and Park occurs in the general population of children, we have a basis for understanding of a wide distribution of structural abnormalities of teeth due to vitamin D deficiency during the period of tooth formation.

If a fair share of children receive suboptimal quantities of vitamin D, it should be possible to demonstrate a reduction in hypoplasia and in caries by addition of the antirachitic vitamin to the dietary of children. Several such studies have indeed claimed this result to occur. Since the first and the best known of these studies were conducted by the Mellanby group, a brief reference to their studies and results will be made.

Although the first study was carried out in Sheffield,[25] it is more convenient to describe the later Birmingham[26] study since the Sheffield study was complicated by several factors with regard to the control and observation of the subjects. In the Birmingham study started in 1928, the children in three orphanages were used as subjects. In so far as was possible to make the assessment, the basal dietary and living conditions in the three homes were practically identical. A special addition was made to the dietary of each home so that there were three groups for observation. These three groups were as follows:

Home 1: Each child received 1 to 1½ ounces treacle (molasses) per day.
Home 2: Each child received 14 to 21 c.c. olive oil per day.
Home 3: Each child received 14 to 21 c.c. of cod-liver oil per day.

A second investigation was later undertaken in one of the homes in which a control group of children received olive oil and an experimental group was given vitamin D as a calciferol dissolved in olive oil. All of the subjects were between the ages of 5 and 14 years and averaged 9.5 years of age. The children continued on the regimen for three years and were examined at six-month intervals. The results of the two investigations are summarized in Figs. 8 to 11.

Fig. 8 shows that the incidence of new caries in teeth erupting after the beginning of the study was definitely less in the cod-liver oil group than in either the treacle or olive oil group. The lower part of the diagram compares the average caries figure in the three groups. The average caries figure is a score which takes into account not only the number of teeth which are carious but also the nature and size of the lesions.

With regard to the original permanent teeth present in the mouth at the beginning of the study, it can be seen (Fig. 9) that although the incidence and spread of caries were less in the cod-liver oil group, the differences cannot be established by the results. It should be indicated that the original permanent teeth of the treacle group were more carious at the beginning of the study than were the teeth of the children in the other two groups.

Fig. 10 refers to Investigation II and shows the over-all change in the proportion of carious teeth and in the average caries figure. These charted data show, at the end of the investigation, that essentially equal percentages of the original permanent teeth were carious in the two groups of children. It will be noted, however, that the children in the vitamin D group had more carious teeth at the beginning of the study than those in the olive oil group. When the data are calculated to show the percentage of originally sound permanent teeth which became carious during the study, the results are much in favor of the vitamin D group. That is, 15.48 per cent of the originally sound teeth in the olive oil group became carious, while only 9.44 per cent of such teeth in the vitamin

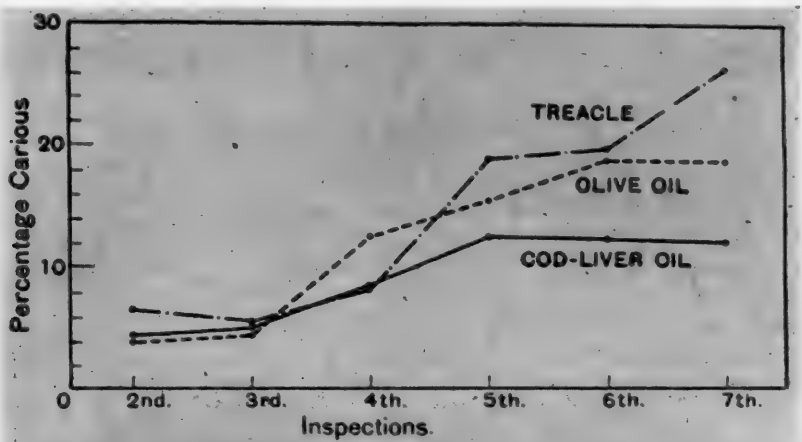

FIG. 1.—Investigation I. Newly-erupted permanent teeth: percentage carious.

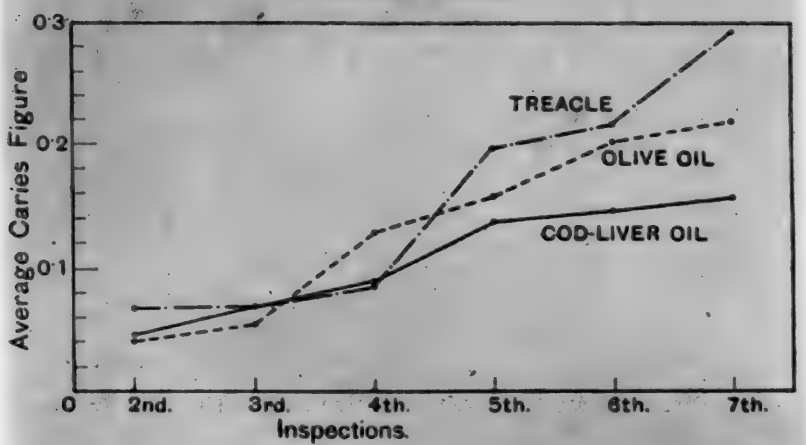

FIG. 2.—Investigation I. Newly-erupted permanent teeth: average caries figure.

Fig. 8.—Investigation I. Newly erupted permanent teeth.

D group were carious at the end of the study. It was, therefore, concluded that significantly less caries both in incidence and spread occurred in the original permanent teeth of the vitamin D group than in the olive oil group. For my part, however, I feel that the original discrepancy in the number of carious teeth in the two groups may have had an important bearing on these results.

Fig. 11 shows the results observed with the teeth which erupted during the course of the second investigation. These results appear to indicate a clear superiority of the teeth in the vitamin D group.

Perhaps the strongest opponent of the value of the antirachitic vitamin in promoting caries resistance is Day, whose observations have been made mainly

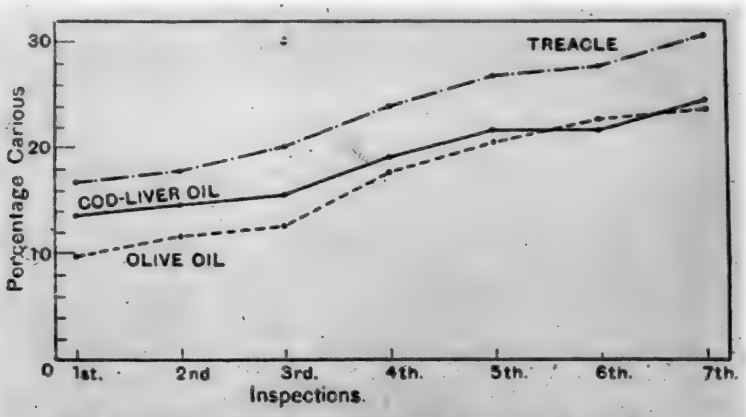

FIG. 3.—Investigation I. Original permanent teeth: percentage carious.

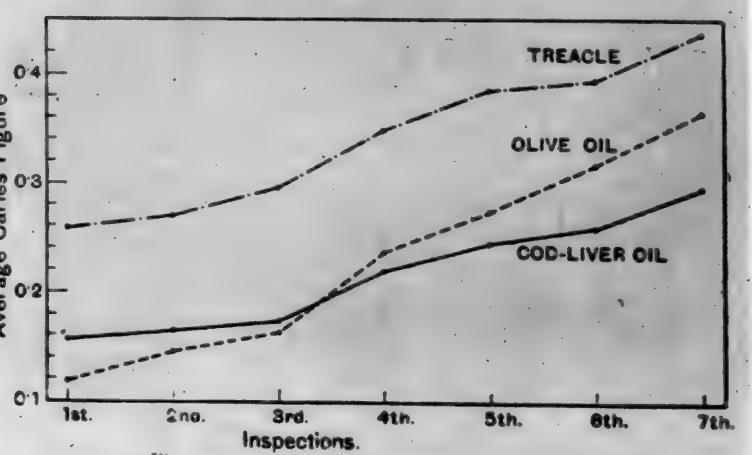

FIG. 4.—Investigation I. Original permanent teeth: average caries figure.

Fig. 9.—Investigation I. Original permanent teeth.

in India. In one study, in Rochester, N. Y., Day and Sedwick[27] gave 40,000 U.S.P. units of vitamin D and 6,000 U.S.P. units of vitamin A daily for fifteen months to 147 children whose average age was 13 years and 10 months. The number of new caries appearing was not significantly different from that occurring in the control group of 171 children of a similar age. It must be said with regard to this study that the dosage of vitamin D employed was massive and on this account may have deleteriously affected the retention of calcium and phosphorus. Also, the subjects studied by Day and Sedwick were at an age at which tooth formation and eruption were more nearly completed than in the children observed by the Mellanby group.

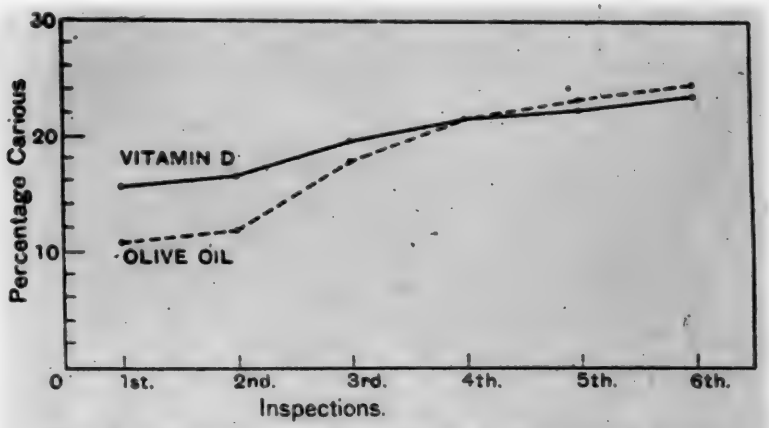

FIG. 5.—Investigation II. Original permanent teeth: percentage carious.

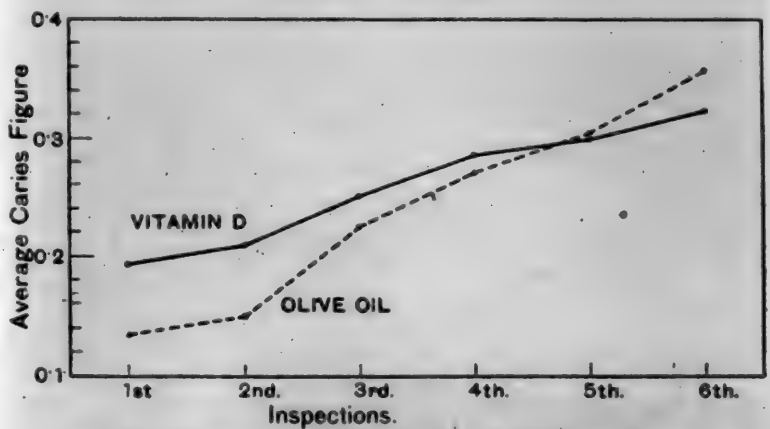

FIG. 6.—Investigation II. Original permanent teeth: average caries figure.

Fig. 10.—Investigation II. Original permanent teeth.

In connection with the influence of age on the effectiveness of vitamin D supplements in the control of dental caries, the work of Anderson, Williams, Halderson, Summerfeldt, and Agnew[21] may be cited. These workers observed a substantial reduction in caries increment in children between the ages of 3 and 10 years, but no effect of vitamin D supplements on the caries increment in children aged 11 to 16 years. There is evidence to support the expected result that when the basal diet contains adequate vitamin D, further supplements of this vitamin are without beneficial effect on the teeth. The paper of Schoenthal and Brodsky[28] may be referred to in this connection.

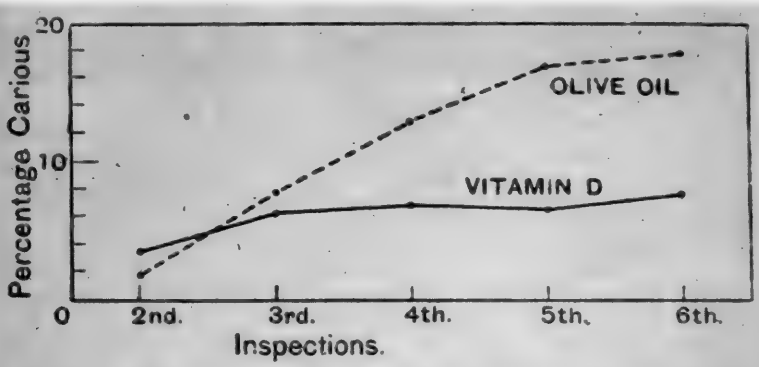

FIG. 7.—Investigation II. Newly-erupted permanent teeth: percentage carious.

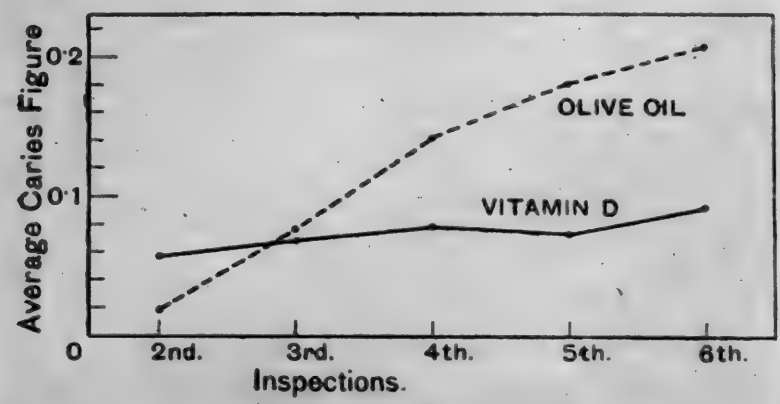

FIG. 8.—Investigation II. Newly-erupted permanent teeth: average caries figure.

Fig. 11.—Investigation II. Newly erupted permanent teeth.

Some further observations of Day may be briefly described because of his contention that there is no correlation between the occurrence of rickets and hypoplasia or between hypoplasia and caries. In one study Day[23] examined 200 Hindu boys of an average age of 14.3 years. These boys were divided into three groups according to whether they exhibited no skeletal signs of past rickets, slight evidence of rickets, or obvious evidence of past rickets (Table I). If anything, those boys with rickets semed to have slightly better teeth than those with no evidence of rickets. It is worthy of mention here that the caries attack rate of all subjects was very low, indeed. The column on the right of Table I shows that no relation of hypoplasia to the presence of stigmata of rickets was assumed by Day. The extremely low figure for caries attack rate observed in Hindu boys is emphasized in Table II, also taken from the paper of Day.

TABLE I*

INCIDENCE OF CARIES AND HYPOPLASIA IN RELATION TO DEGREE OF CLINICAL RICKETS

DEGREE OF CLINICAL RICKETS	NO. OF BOYS EXAMINED	NO. OF TEETH PRESENT	NO. OF CAVITIES PRESENT	CARIES (PER CENT)	AVERAGE NO. CAVITIES PER MOUTH	HYPOPLASIA (NUMBER)	HYPOPLASIA (PER CENT)
A. No clinical signs of rickets	113	3,019	172	5.69	1.52	76	67.26
B. Slight clinical rickets	64	1,782	109	6.12	1.70	40	62.50
C. Moderate to severe clinical rickets	23	656	32	4.88	1.39	14	60.87
Total	200	5,457	313	5.74	1.56	130	65.00

*From C. D. M. Day: *Brit. Dent. J.* **76**: 143, 1944.

Table II compares the caries attack rate of three groups: the 200 boys just referred to, a group of Lahore city children in India, and a group of children in Rochester, New York. Note how very much greater is the caries attack rate of the American children than that observed in either the Lahore or Kangra Valley children. These data are presented in order to indicate that the lack of correlation between rickets on one hand and hypoplasia and caries attack on the other hand observed by Day may have been due to the operation of some powerful caries protective factor in India which is absent in American and English children.

TABLE II*

COMPARISON OF CARIES INCIDENCE IN VARIOUS GROUPS

LOCALITY	NO. OF CHILDREN	AVERAGE AGE	AVERAGE NO. OF CAVITIES PER MOUTH	PER CENT TEETH CARIOUS	PER CENT CHILDREN CARIES-FREE
Kangra Valley	200	14.33	1.56	5.74	49.0
Lahore City, India	756	13.58	5.74	17.66	5.95
Rochester, New York	500	12.94	21.76	57.00	0.20

*C. D. M. Day: *Brit. Dent. J.* **76**: 145, 1944.

My digest of the studies relating caries susceptibility to vitamin D intake leads me to the following opinions: (1) Teeth formed during a period of suboptimal intake, all other conditions being equal, possess an increased susceptibility to caries. (2) If the intake of vitamin D is adequate and of the order of

400 to 800 International units per day, further supplements with this vitamin are not likely to be of value. (3) Vitamin D supplements do not appreciably affect the initiation or progress of caries in teeth already erupted.

There remains now only sufficient time barely to mention the role of the other vitamins and the minerals in the control of caries. With regard to vitamin A, it can be stated that there is no evidence that deficiencies of this vitamin either during the period of tooth formation or at a later period contribute to caries susceptibility.[29] No studies have been reported of the effect of inadequate amounts of members of the vitamin B complex during the time of tooth formation on caries experience of man or animals. Kniesner, Mann, and Spies[30] reported that the caries incidence of patients hospitalized for treatment of vitamin B complex deficiencies was apparently not increased.

A considerable effort has been expended to demonstrate whether vitamin C deficiencies result in any significant effect on the teeth as regards their resistance to caries. The often quoted study of Hanke[31] carried out in an orphanage at Moosehart, Illinois, presented a striking reduction in caries susceptibility in a group of children who were each given for one year a pint of orange juice and the juice of one lemon per day. The interpretation of this study is exceedingly difficult. Further difficulty in the understanding of this study is experienced because it was reported that three ounces of orange juice per day per child, which would be expected to contain something like 50 mg. of ascorbic acid, failed to maintain the high caries resistance observed when the subjects were receiving one pint of orange juice per day.

Grandison, Stott, and Cruickshank,[32] who gave daily supplements of 200 mg. of ascorbic acid, actually observed the development of more caries than in a control group. While it should not be concluded that the ascorbic acid was actually harmful on account of differences between the composition of the experimental and control groups, the fact remains that no beneficial effects were noted.

With regard to the minerals, there are no data which indicate any increase in the dental caries experience of children receiving diets partially deficient in calcium or phosphorus or both. Supplementation of a diet moderately low in calcium and phosphorus with additional amounts of these minerals did not result, in the hands of Malan and Ockerse,[33] in any significant reduction in caries increment of ninety-seven children over a three-year period. The claims of Harootian[34] in this connection need not be further discussed.

Finally, I should like to state that I have no doubt that there may be individual differences in metabolic efficiency which permit of differences in mineral retention correlated with caries susceptibility. Practically all of the work on this topic has been carried out by the Iowa school and will undoubtedly be mentioned by Dr. Stearns.

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DISCUSSION

DR. EASLICK.—Thank you very much. Dr. Armstrong's presentation is open for discussion. Dr. Stearns?

DR. GENEVIEVE STEARNS.—I have a question that I should like to ask Dr. Armstrong. About a year ago a Dutch dentist named Smith was sent to this country by the Netherlands Government to pick up the new work in the field of dental caries. While visiting our university he made a statement which made a very great impression on me. Dr. Smith lived in what was called the "hunger" area of Holland, that is, the area that was not only occupied by the Germans all through the war, but during the latter part of the war was subjected to terrific starvation. The people were down to 600 calories a day for the last four or five months of the war. He commented, as have many others, that there was surprisingly little dental caries among his patients and those of other dentists, even during the months of the "hunger" period. And then, he said, the American troops came in and generously shared their "K" rations with the Dutch people. He said, "The American hardtack tasted, oh, so good to us because we were so hungry and we ate the awful rock. The next week and from then on my office, and the offices of every other dentist, was full of patients, not with caries but with fractured teeth." He said he had never, nor had any other dentist in the city, seen anything like this incidence of fractured teeth. The American soldiers did not have fractured teeth, and they had to eat the hardtack when they had nothing else. If it is impossible to change the structure of the teeth, how would you account for this marked incidence of fractured teeth after a long period of undernutrition?

DR. EASLICK.—Do you want to take that, Dr. Armstrong, or refer it to a dentist?

DR. ARMSTRONG.—We have now heard, with your report, of three instances of a people in the war-torn countries having a lower incidence of caries than during the immediately preceding years of peace. The observations of Toverud in Norway constitute one such instance and a report from London, by Lady Mellanby, I believe, showed an improvement in the caries attack rate in school children in that city during the war. I suppose that some people would suggest that the relative lack of refined carbohydrate in the dietary of the people of several European countries during the war years would account for these findings.

I don't know how to explain the increased number of teeth fractured by people in Holland when they were given hardtack. As a matter of fact, I did not have the impression that the biscuit in the "K" ration was particularly hard. As a guess, I suggest the possibility that the Dutch had never seen bread of this sort. As you said, Dr. Stearns, they were ravenously hungry and it may be that their mastication was overly vigorous because of their lack of experience as to how to deal with the material.

DR. EASLICK.—Dr. Casey?

DR. GERARD J. CASEY.—As a member of the European expeditionary force may I add a word about the "K" ration, possibly in explanation for the fracture of teeth, because I broke one myself over there. I was eating "K" rations at the time. I think generally in European countries the dentists do extensive root canal work. I may be corrected, but from my impressions of dental offices in Gutenberg that I have gone into looking for things that might be of interest, the Dutch dentists seemed to do extensive root canal work. If there are large fillings like MOD's to be placed in teeth, they have the root filled and when the patient starts chewing those crackers the teeth may break. Since the teeth are pulpless they are more fragile. I had a pulpless tooth, and I broke a large MOD. So it may be that the Dutch people had many pulpless teeth with fillings, and that is why the teeth fractured on those crackers.

DR. EASLICK.—Thanks, Dr. Casey. Dr. Atkins?

DR. A. PAUL ATKINS.—This may not be pertinent, but I have a question I should like to ask the doctor as long as he said that the administration of vitamin D was perfectly safe in the amounts that we use. I know that it is, but as long as mothers can go to the drug store and buy vitamin D and everything else by the carload and get their kid in the corner and feed him all they want without any justification or knowledge of how much or why, does Dr. Armstrong care to say a word of caution about overdosage of vitamin D?

DR. ARMSTRONG.—Your point is one which well may be discussed. The vitamin D content of cod-liver oil is so low that I don't believe you could persuade any infant to take enough to cause vitamin D intoxication. The more potent preparations, which are prepared by adding calciferol to either olive oil or cod-liver oil, are another matter. However, with regard to all forms of medication, we must recognize our duty to explain carefully the dosage to be used. The amounts of vitamin D required for toxic effects in the adult are pretty high and would be difficult to obtain by accident. Such cases have mainly come from the use of extremely large doses of vitamin D in the treatment of arthritis. While you have a point, I think that if we stick to the use of cod-liver oil the dangers are not very great.

DR. EASLICK.—Dr. Bibby?

DR. BASIL G. BIBBY.—I should like to make a point relative to Mellanby's work. Her last report—with Miss Kumulus, I think that is the name—has retracted somewhat their basic conclusion regarding the vitamin D effects. They made a study of orphanages, public schools, and county schools and decided, after spending a little while in each institution, that there was a relationship between the degree of microscopic hypoplasia and the amount of dental caries that did not hold from institution to institution. Their present stand is that there must be some factor other than microscopic hypoplasia which predisposes to dental caries, which I think is a significant conclusion coming from that source.

DR. ARMSTRONG.—Yes, it is indeed.

DR. BIBBY.—Yes. The other thing I should like to ask Dr. Armstrong is, at the A. D. A. meeting in Chicago Dr. Shaw, a reliable worker, claims to have increased caries in animals to as much as fivefold by feeding carbohydrate diets to the mother and to the animals before the eruption of the teeth. I wonder if you would like to explain that one?

DR. ARMSTRONG.—No!

DR. EASLICK.—Dr. Taylor?

DR. EDWARD TAYLOR.—I should like to ask Dr. Armstrong if he would elaborate somewhat on the importance of sunshine, the sunshine hours, and the quality of sunshine as in relation to vitamin D.

DR. ARMSTRONG.—I wish Dr. East or Dr. Mills were here to take that question. I think it is pretty clear that there is a difference in the geographical incidence of caries. As we go from north to south the number of cavities becomes fewer, and one might suppose that it is because there are fewer clouds in the south than in the north, and that the latitude of the south is more favorably disposed for sunshine, and so on. We have a source for production of vitamin D in our own skins, a derivative of cholesterol, 7-dehydrocholesterol, which will upon radiation with ultraviolet light be converted into the same vitamin D that occurs in cod-liver oil. That individual who receives more ultraviolet radiation from the sun or artificial ultraviolet light requires correspondingly less vitamin D in his food or medication. Now, as an actual matter of fact—perhaps Dr. Stearns may have more information than I do—I know of no indication that the adult requires vitamin D, that is, vitamin D in his food. Even the amount of vitamin D that we can get simply by radiation of our hands and face, even if we never go swimming, is apparently sufficient to maintain the average or normal adult in good health. Now, true enough, osteomalacia is due to a lack of vitamin D, but due also to a reduced calcium intake and to repeated pregnancies and lactation. Let's say men (and women who are pregnant only occasionally) seem not to need any increased amounts of vitamin D above that which is present in a normal or average diet.

DR. EASLICK.—I think that some other discussion may come out after the next speaker, and I should like to introduce the next speaker now, because the time has come, gentlemen, to listen to the feminine part of this faculty. Before I introduce her, I think that you are entitled, in all justice, to some background from the early stages of the development of this Workshop. The original invitation to discuss the adequacy of the diet as a caries control measure went to Dr. Philip Jeans of the University of Iowa. When he hesitated to become involved in a controversial subject, the suggestion was made that Dr. Julian Boyd present his viewpoint. Dr. Boyd was going on a vacation. In a bit of desperation, I wrote Dr. Jeans requesting that he help me by naming his choice for this morning's presentation. His letter came back suggesting Dr. Genevieve Stearns of his department of University Hospitals. I then called Dr. Howard B. Lewis, Chairman of the

Division of Biochemistry of the University of Michigan, and asked Dr. Lewis, "How will this lady from Iowa perform in an otherwise all-male faculty?" Replied Dr. Lewis, "If Genevieve Stearns could struggle through our graduate curriculum and come out with a Ph.D. degree all tied up in maize and blue ribbon, she can take care of herself in any all-male faculty!" We signed her.

Now, Dr. Genevieve Stearns has been asked to present the subject, "The Effectiveness of an Adequate Diet in the Control of Dental Caries." She now is a Professor of Biochemistry at the University of Iowa and, for me, Dr. Stearns, it is a pleasure to present an alumna of the University of Michigan behind whose skirts two husky male Hawkeyes hid when my Planning Committee was seeking speakers. That's your introduction.

THE EFFECTIVENESS OF AN ADEQUATE DIET IN THE CONTROL OF DENTAL CARIES

GENEVIEVE STEARNS, PH.D., RESEARCH PROFESSOR OF PEDIATRICS, UNIVERSITY OF IOWA COLLEGE OF MEDICINE, IOWA CITY, IOWA

It is obvious to us all that push-button control of dental caries has not yet been achieved, and never will be. Dental caries is a complex problem which probably will not be solved by any one method of control. So as a public health measure, it is worth while to examine all of the evidence, even that concerning which some of you are most skeptical.

Dental caries is a very common trouble; poor nutrition also is far too common. These two observations are not necessarily related, but whatever the causes of dental caries, they must be widespread among the American public.

If nutrition is related to dental caries, what nutritional factors are most likely to be involved, and how are they involved? Are certain periods of life more likely to be periods of nutritional stress than others? If so, when do these danger periods occur? In what manner does the nutritional state affect the production of dental caries?

The last question is very simply answered: We don't know. Dr. Armstrong has just told us that in his opinion any factors acting to inhibit dental caries must act through the oral environment. I have great respect for Dr. Armstrong's opinion. We can say, then, that it seems probable that if nutrition affects the incidence of dental caries, it does so through some action on the oral environment. The known association between heavy calculus and freedom from caries lends point to this view. But the fact that we do not understand fully how nutrition might act does not mean it has no effect; we all have learned that changes too minute for us to measure today may become tremendously important tomorrow after we develop methods sufficiently accurate to study them.

The data summarized in the following discussion are drawn largely from our own findings at Iowa, supplemented by the excellent studies of Dr. Icie Macy Hoobler and her collaborators at the Children's Fund of Michigan and the findings of the Midwestern College study of the nutrition of college women.

For purposes of this discussion, the nutrients of the diet may be separated into two classes: (1) those whose digestion products are easily soluble in water, and (2) those soluble with difficulty. Those products easily soluble in water are usually well absorbed. If eaten, they are absorbed and used by the body. This group includes the proteins, carbohydrates, water-soluble vitamins, and the readily soluble salts, such as those of sodium and potassium. Of this group of nutrients, we are concerned only with the nutrient likely to be eaten in insufficient quantity—the proteins. The second group includes substances absorbed with some difficulty at best and which, with relatively small changes in nutritional state, may be absorbed very poorly, or not at all. The substances of the second group are the fats and the vitamins carried by fats, vitamins A, D, E, and K. It includes also the less soluble polyvalent minerals, calcium, phosphorus, and iron. For these substances we cannot assume that intake is equivalent to absorption and utilization. The intake may be generous; the

absorption, zero. It seems probable, then, that in a study of relationship of nutrition and dental caries, we should be concerned primarily with the substances of this second group of nutrients, those absorbed with difficulty. If periods of nutritional stress do occur, we should expect decreased absorption and retention of these difficultly handled substances, whereas nutrients easily absorbed would be little affected.

When do these periods of stress occur? The first is the period of infancy. The baby absorbs calcium and phosphorus of cow's milk poorly and develops rickets unless vitamin D is given. The nutrition during infancy is important in the prevention of dental caries, for, as Dr. Armstrong has just said, other things being equal, a well-formed tooth decays probably less easily than a poorly formed one, and calcification of the permanent teeth begins during infancy. So we assume that all babies in this country need vitamin D in addition to whatever sunlight they get. We have learned that the frail baby, or the baby with even a mild chronic illness, does not absorb fat well; therefore he will not absorb much of the fat-carried vitamins of the diet (vitamins A, D, E, and K). Thus the vitamin D metabolism is affected; calcium, phosphorus, and iron are absorbed very poorly and may even be lost from the body in greater amounts than those ingested.

A baby who has a mild but chronic infection is far more likely to become anemic than a baby who has one severe but short illness. What is true of iron is true also of calcium and phosphorus, though these deficiencies will not be obvious unless sufficiently severe to cause rickets. Poor structural development of teeth may thus be expected in babies whose nutrition is poor, in those who are subject to frequent colds or who have chronic infections, even though these are relatively mild.

The development of the permanent teeth continues through early childhood. Although parents generally have become educated into giving infants good diets including vitamin D daily, after the child is able to feed himself entirely his nutrition is likely to suffer. Because rickets is uncommon after the period of infancy, few children over 2 years old are given vitamin D. Yet children of this age group are inefficient in retaining calcium and phosphorus unless vitamin D is given also.

We have studied the retention of calcium and phosphorus by children of all ages. Without added vitamin D, a few children show adequate retention, a few show no retention or even loss of these minerals, and the majority show some retention though the amount often is inadequate for good bone and tooth development. When 300 to 400 units of vitamin D are added to the daily diet, the few children whose retention was excellent will show little or no increase in calcium and phosphorus retention. The children whose retention was very poor will show a marked increase in retention. The addition of vitamin D to the dietary results in greatly improved nutrition of the group by bringing up the level of nutrition of all more nearly to the level of those most efficient without the added vitamin D.

We have found that as the children grow there is some tendency to increased efficiency of absorption without added vitamin D when the milk intake

is generous, three-fourths to one quart daily. Possibly, their increased amount of outdoor play provides enough vitamin from the sun's rays. However, in each group of children we have studied, we have found some children unable to maintain an adequate retention without added vitamin D despite an ample milk intake. Therefore we have concluded that it is wise to give all children a moderate amount of vitamin D (300-400 U.S.P. units daily) and three-fourths to one quart of milk daily during the entire period of growth. Such a provision is good insurance, at least for the development of sound teeth, and possibly for their preservation.

At any age during childhood, illness results in lowered gastrointestinal efficiency, just as it did in infancy. During these periods, absorption of iron, calcium, phosphorus, fats, and the fat-soluble vitamins is greatly decreased.

Adolescence is a second period of metabolic stress. Infancy and early childhood constituted the first such period, with the children showing increased efficiency with increasing age up to the period of the prepubertal growth spurt.

The need for calcium is not constant throughout childhood; it varies with the rate of growth normal for the child at each age. It is obvious that a daily pint of milk does not provide enough calcium for those over 5 years old, even when vitamin D is given in addition. One and one-half pints of milk daily together with 350 U.S.P. units of vitamin D permit ample retention by all, and for the majority, allow for storage of calcium for the inevitable emergencies that occur with illness when no calcium and phosphorus are retained.

The estimated daily calcium need during the period of very rapid growth is high, 400 to 500 mg. of calcium daily. The average child does not maintain such a retention, for the bones become progressively poorer in mineral. Todd called this skeletal condition "the physiological osteoporosis of adolescence." Our problem was to determine whether this osteoporosis were truly physiological or the result of undernutrition.

The first group studied was composed of eight girls, from 11 to 15 years old, who had lived in an orphanage for from three months to eight years. We gave these girls graded amounts of milk and of vitamin D in an attempt to find the level of intake necessary to maintain adequate retention. When no vitamin D was given, the lowest retention was 10 mg. calcium daily—$\frac{1}{40}$ of the need. As we added vitamin D and increased the milk intake, retention values tended to increase, but even though the girls drank nearly two quarts of milk daily and had 800 U.S.P. units of vitamin D each day, the average retention of the group was well below the need. A second study of a group of eight boys from the same orphanage gave essentially the same results. Why should these children, at the time of their greatest need of minerals, be unable to retain even as much as younger children whose need is less? J. A. Johnston of Detroit, has found that on or about the time of onset of menstruation, girls show a decreased ability to utilize calcium and phosphorus. In our study, the boys retained these minerals no better than the girls. We could not explain satisfactorily these unexpected findings.

A third study was undertaken with a group of girls from a second orphanage. This institution was smaller and had a larger food budget per

child than had the first orphanage. The girls were in abundant health and appeared more vigorous, more mentally alert than the first group. The study followed the pattern of the previous studies. The retention of calcium and phosphorus was studied with increasing levels of milk and of vitamin D. Contrary to the previous studies, these girls showed ample retention when given one quart of milk and 400 units of vitamin D daily along with an otherwise good diet.

Why was this group of girls able to retain larger amounts of calcium and phosphorus than the subjects in the first two groups who had been given twice the intake of milk and of vitamin D? For at least five years the "good" group had lived at the orphanage and their nutrition had been excellent during all this time. The poorer groups, both boys and girls, had lived for several years under poorer nutritional conditions at their orphanage. The only exception was a 14-year-old girl who had lived in a good home, under excellent care, until three months prior to our study. Her retention values were the highest in her group and approached those of the "good group." From these findings we drew the tentative conclusion that the previous nutritional state of the child has a notable influence on the efficiency of utilization of a good diet.

To test this hypothesis, a third group of 11- to 17-year-old girls was studied. This time each girl was given a good diet including one quart of milk and 400 units of vitamin D daily. The calcium and phosphorus retention values were determined for fifteen days each month. The retentions the first month were only fair; improvement in retention, without any change of intake level, continued until by the sixth month all the girls under 15 years of age were retaining ample amounts of calcium and phosphorus. The older girls (15 to 17 years) never achieved the expected retention during the eight months of the study. We have concluded, therefore, that the longer a child is on a dietary regimen that is not fully adequate, the poorer is the condition of his gastrointestinal tract, and the child will absorb poorly those nutrients which are difficultly soluble—calcium, phosphorus, iron, the fat-soluble vitamins, and fat. It seems important to us that the dental condition of the girls in the best nourished group was superior to that of the more poorly nourished groups. It is also important to remember that the latter groups were within normal range of height and weight for age though far inferior in nutrition. When these poorer groups were given a good diet, the effects, if any, on incidence of dental caries could not possibly become evident for many, many months.

A third period of nutritional stress is lactation, but not pregnancy. All the studies of women in pregnancy tend to show that with a good diet, including a moderate amount of vitamin D, the average pregnant woman can retain sufficient calcium for herself and her baby, and often store a little for later need.

For some reason as yet not clear, it is much harder for a woman to maintain calcium-phosphorus balance in lactation than during her pregnancy, even though her diet is ample and the amount of calcium and phosphorus secreted in the milk is no more than the amount taken up by the growing fetus. The average woman loses both calcium and phosphorus from her body during lactation unless she takes one and one-half quart or more milk daily, with a moderate amount of vitamin D.

I know of no studies of the effect of lactation on the dental caries rate. Many such studies have been reported for pregancy with no correlation observed. The old saying, "A tooth for every child" was coined in the period when practically all babies were breast fed. Why in its modern interpretation it has been related exclusively to pregnancy is difficult to understand. Very little is required of a pregnant woman in the way of storage of calcium and phosphorus for the fetus until the last three months of pregnancy; nearly half the baby's calcium is stored in the last six weeks of gestation. In lactation, the drain is immediate and continuing; the mother appears to be less efficient in absorbing and retaining calcium than she was during pregnancy. It seems that a study of the effect of lactation on dental caries may be far more illuminating than studies of pregnancy and dental caries. Effects of pregnancy on caries would show up later than these studies have been carried.

A fourth period of nutritional stress in the life of a woman comes at menopause. Apparently at this time many women show a decreased gastrointestinal efficiency; such women absorb calcium, phosphorus, iron, fats, and fat-soluble vitamins poorly, if at all. They arrive at this period after years of poor dietary intake and the drain of several pregnancies and lactation periods. Anemia is common; osteoporosis is very common. Women coming to the hospital at this age because of osteoporosis usually are edentulous, and so correlation between dental caries rate and this period of stress is not possible.

To return to our original thesis, if nutrition is related to dental caries (and I should expect such relation to be inverse) the better the nutrition, the less the decay. Then we should expect increasing attack rates during these periods of nutritional stress outlined. There is some evidence of such increase with the onset of rapid growth in early adolescence. The caries attack rates should be studied also during lactation and postlactation periods and in menopause. Such studies would no much to clarify our thinking.

Many of the studies at present in the literature are difficult, if not impossible, to interpret. Often one factor of the diet is changed; no attention is paid to the remaining factors. If the diet is grossly inadequate in many factors, improving it in one factor only cannot possibly be effective. Many of the studies on vitamin D are of this type. Vitamin D acts by increasing the absorption of calcium and phosphorus from the gastrointestinal tract. If sufficient calcium and phosphorus are not in the tract to be absorbed, no amount of vitamin D can be effective. Yet vitamin D has been given to school children with no check on their calcium and phosphorus intake, and far-reaching conclusions have been drawn from the results. In many school districts in poorer urban sections of our large cities, milk sales show that few children get over a cup of milk daily. That amount is not sufficient for "wear-and-tear" or necessary excretion. No amount of vitamin D can cause retention with so low an intake. Our own studies have shown that significant increases in calcium retention with administration of vitamin D are obtained only when the milk intake is one point (500 c.c.) or more daily.

Another error in many reports of the effect of nutrition and dental caries is the common error of changing several factors but ascribing the results to one

factor only. To say, "We took away all sugar and substituted milk and eggs, meat and vegetables. Because of the reduction in carbohydrate, a reduction in caries was observed." Perhaps the statement is correct, but it is not proved. Howe's group in Boston studied carefully the caries rate of 200 children whose diets were carefully regulated to contain ample amounts of all known nutritional essentials. Beyond these factors, the diet was free choice—not sugar free. They observed a significant reduction in dental caries.

At Iowa we have two groups of children under careful dietary control. Each group gets an excellent dietary regimen. The diabetic children get no free sugar; the children with celiac disease may get 60 per cent of their calories as glucose (dextrose) which is given in 10 per cent solution flavored with fruit juice. Diets of both groups are controlled for several years. Both groups remain almost caries free. Is it something in the disease process that prevents caries? We think not. Poorly cared-for children with either disease may have rampant caries. I once saw an 18-month-old child with celiac disease and every tooth that had erupted was decayed to the gum line. The last time I saw her she was 10 years old and completely free from dental caries. Treatment was begun at 18 months and the disease had been well controlled.

In a hospital one can study single children or small groups of children over a period of many months. They are under close supervision at all times. These carefully controlled studies lead the way for the survey studies of larger groups which are necessarily under loose control. The small studies show what can happen; the larger studies show what you can expect in the way of cooperation by the general public. One such careful study made in our hospital concerned a 12-year-old girl with active dental caries. The cavities were untouched; the child was not given a toothbrush. Her diet was nutritionally adequate and contained as much free sugar and jam as we could get her to take. Our water supply has only 0.1 ppm fluorine. At the end of eight months she showed no extension of caries.

On the other side of the picture is the 14-year-old girl brought to us because of rampant caries. She had had no cavities until six months before admission, but during that six months had developed twenty-eight cavities. She had always had an excellent diet. Retention studies showed no retention of calcium and phosphorus. Vitamin D added to the diet was without effect. If vitamin D was ineffective, we reasoned that she probably did not absorb any of the fat-soluble vitamins. Checking her vitamin A status with the dark adaptation tests, we found it very poor, though for a month preceding the test she had received 20,000 I.U. of vitamin A daily in addition to that of her diet. We then gave her a diet low in fat, but ample nutritionally, and with added vitamins A and D and bile salt to enhance the utilization of the fat-carried vitamins. Within a few days, her vitamin A status was normal and she began retaining calcium and phosphorus. Within a short time the exposed dentin hardened and the girl showed no further carious activity.

These two case histories are illustrative of several such studies we have conducted wherein caries has been arrested under diet therapy with diets high in

sugar, and wherein caries has been arrested after improvement in the utilization of the diet. We have never failed to achieve arrest of caries.

SUMMARY

To summarize, if the incidence of caries is related to the state of nutrition, we should expect the greatest incidence of caries during or shortly after periods of nutritional stress. Such periods occur at any age during illness, especially during chronic illnesses, though they be low-grade infections. Other known periods of stresses are: (1) the periods of rapid growth preceding puberty, (2) lactation, and (3) menopause. The incidence of dental caries is usually considered to be increased at about puberty. The incidence at the other periods of stress could well be studied.

In evaluating nutritional studies in relation to dental caries it is important to know the nutritional history of the subjects. Improvement of the diet in one respect only cannot be expected to bring improvement if the diet as a whole remains hopelessly below standard, or if the chief deficiency is other than the material supplied. The effectiveness of vitamin D, in particular, cannot be determined unless it is proved that the calcium and phosphorus intake is sufficiently high to permit an increase in retention through the action of the vitamin.

When several factors of the dietary regimen have been altered, results cannot be ascribed to only one factor. That seems so obvious as to be trite, but still it is the most common error in research reports.

At the State University of Iowa, keeping children under strictly controlled dietary regimens, we have observed complete arrest of caries with diets high in sugar, and with diets low in sugar but always nutritionally adequate and well-absorbed. From our nutritional studies on normal children, we have concluded that the longer a child remains in a state of poor nutrition, the longer it takes for him to utilize a good diet when he eats it. Often many months must elapse before the child becomes well nourished. Under these conditions, it is slight wonder that many investigators feel that diet is not a major factor in the prevention of dental caries. It is not diet, but the state of nutrition which we believe to be an important factor in the incidence of dental caries.

DISCUSSION

DR. EASLICK.—Now, we are only about a half hour behind here at the moment. Let's have a brief discussion of Dr. Stearns' presentation.

DR. HAROLD J. NOYES (University of Oregon Dental School).—Dr. Stearns, there are two points that I should like to discuss. First, briefly, is the very interesting finding which Dr. Stearns mentioned of the period of lactation being a serious time in nutrition. I was very interested some years ago in Dr. Dobb's laboratory to have him point out, when we were looking at x-rays, that it was the period of lactation and not pregnancy in which there was mineral evidence of a difficulty in storing and actually a loss of mineral and that happened irrespective of whether the mother nurses her baby or not, which to me was a particularly interesting feature, and I have been glad to have some backing in the contention, which has been made for some time, that pregnancy is not the time but it was the time of lactation, if at all.

The second is that I am one of these unique individuals who do believe that we have knowledge of the mechanism of dental caries. I think that much of our confusion in that is that we have had a tendency to attribute it to a single individual factor instead of to the mechanism. If that is so, then we would not expect any single dietary factor, or even diet in itself except in so far as it affects the mechanism, to be effective. The portion of that mechanism which can definitely be affected is the structure of the tooth, the arrangement of teeth to a certain and limited extent, and the fluids of the mouth. In so far as we can affect, through a sound nutritional program, the mechanism of dental caries, then we will have that much contribution coming from the over-all picture of caries. Isn't it a little bit the same as your own nutritional problem? No single factor is the answer in nutrition, no single factor other than that you can break the chain in the mechanism of caries, and any factor which breaks the chain in the carious mechanism will have an effect on dental caries?

DR. STEARNS.—I agree wholeheartedly with Dr. Noyes.

MISS ADELIA M. BEEUWKES (University of Michigan).—I should like to bring out one point, and that is, I don't think you are quite justified, Dr. Stearns, in suggesting that we are changing the diets of all of our patients from an inadequate to an adequate diet, because in many instances our patients are on a diet that you and I would consider adequate. The change is the reduction in carbohydrates rather than the change from an inadequate to an adequate diet. In many instances we would have one specific change that you asked for experimentally.

DR. STEARNS.—Is your diet adequate in all respects? That is, these children, you know, have ingested a diet adequate in all respects?

MISS BEEUWKES.—Well, Dr. Stearns, it is adequate in so far as either you or I could ascertain from long and tedious computation with the mothers as well as with the children, not by going into the home for a quantitative study.

DR. STEARNS.—Of course, that is the great difficulty of any outpatient study. You never have the final check. We have found that many of our mothers were honest as could be; they were offering their children a very adequate diet and the children were not eating it. That finding is particularly true, I think, in school children who stop in at the corner drugstore on the way home, because that is their crowd's meeting place, and they come home with very little appetite. They are offered an adequate diet, but the amounts that are eaten are grossly inadequate. That could be a large factor.

DR. EASLICK.—Ladies and gentlemen, for the final speaker in this area, the dietary control of caries, and for the final formal speaker of this Workshop, the Planning Committee went further and came back with more—because they went clear to California and came back with the 220-pound Hermann Becks.

DR. BECKS.—218!

DR. EASLICK.—Down to 218 because of his hard work in this Workshop! Dr. Becks, we welcome this reversal of the young-man-go-west formula and enjoy the old-man-come-east.

Dr. Becks is Professor of Dental Medicine at the School of Dentistry of the University of California. The subject assigned to him is twofold in nature, "The Physical Consistency of Food and the Restriction of Refined Carbohydrates in the Diet—The Effect of Each on Caries." Dr. Becks, don't be discouraged because we ran overtime; we are going to curb a lot of appetites and give you all of the time that you need to present your subject, and that is your introduction.

17 9

THE PHYSICAL CONSISTENCY OF FOOD AND REFINED CARBOHYDRATE RESTRICTIONS—THEIR EFFECT ON CARIES

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This last report of the Dental Caries Work Shop on "The Physical Consistency of Food and Refined Carbohydrate Restrictions—Their Effect on Caries" must be short by necessity because it constitutes a summary of established and widely accepted facts which have repeatedly appeared in the dental and medical literature by the Michigan and California schools and other laboratories. These facts speak so overwhelmingly in favor of the use of the oral *Lactobacillus acidophilus* index for the determination of caries activity and inactivity that it is almost inconceivable to accept future results in the field of dental caries research if they are not augmented by a quantitative estimate of acidogenic microorganisms.

Dr. Hine has already discussed the effect of detergent foods as cleansing agents and has referred to the publication of Knighton, who showed by a specially developed method that after chewing a cake of yeast a reduction in the number of oral bacteria was possible by means of chewing gum, apples, paraffin, oranges, bananas, candy bars, tooth brushing, etc. The other studies cited by Dr. Hine indicate that the number of acidogenic microorganisms may possibly be influenced by the use of detergent foods; however, it has been our experience that if actual rampant caries activity exists the influence of detergent foods is minimal but, on the other hand, most difficult to measure accurately.

Since the general theme of this meeting has been The Evaluation of "Dental Caries Control Technics," I shall confine myself for the main part to a practical measure of control and prevention of dental caries, namely, by means of carbohydrate reduction. The studies which have been conducted at the University of California for the last ten years[1, 2, 5] have been primarily in the nature of a clinical investigation to test on a broad basis the original concept of the Michigan school, that a reduction of caries activity can be accomplished by reducing an excessive refined carbohydrate intake, which in turn causes the number of lactobacilli to decrease. Our studies were in no way aimed at a search for the etiology of dental caries.

Keeping in mind that from a practical point of view the two avenues of (1) individual and (2) mass control or prevention may be followed, the reduction of refined sugars is strictly an individual control or prevention program and naturally a difficult one. It is quite obvious that any attempt on the part of dentists to reduce refined carbohydrates and sugars in the daily food intake of their patients requires that they have a thorough understanding of foods and the many problems of nutrition. On the other hand, it does not seem reasonable to apply this somewhat complicated procedure to patients who develop only one, two, or three cavities per annum. In fact, it is our belief that this procedure should apply only to rampant dental caries cases in which the life expectancy

From the George Williams Hooper Foundation for Medical Research and the Division of Dental Medicine, College of Dentistry, University of California.

of the denture, because of the development of many cavities, is greatly endangered. Therefore, in more mitigated forms of dental caries where only an occasional cavity develops it might be sufficient to advise a patient to reduce the refined sugar intake to a minimum without running concurrent *L. acidophilus* tests. However, in the extreme forms of dental caries the change of the *L. acidophilus* index as the result of a reduced sugar intake should be watched carefully.

The first assumption that the intake of excessive sugars, and especially refined sugars, must be related to dental caries probably was not an original discovery with Bunting and Jay and their collaborators[3, 4, 6-13] because it is a widely understood observation that the excessive use of sugars is detrimental to the teeth. However, it was left to the Michigan school to provide sound clinical observations, a laboratory test, and judgment to show that a trias exists in the process of the development of caries: (1) at the time of the development of dental caries, (2) a large number of oral lactobacilli is present and one finds in the overwhelming majority that (3) the host is consuming large quantities of sugar. The reader should be cautioned with regard to an interpretation of this statement since no attempt is being made and no data are available in this laboratory which would definitely establish a causative reltionship between lactobacilli, sugar, and dental caries even though the clinical impression is overwhelming.

After the first observations on this subject were published by the Michigan group, which were a challenge to the dental profession and the public, it was decided to put the observations of Bunting and Jay to a clinical test and to establish on a broad basis and on a large number of patients (1) whether the findings could be confirmed and were valid for various age groups and (2) whether they were practical to apply in a general dental practice. For a working plan three objectives were set up, to establish: (1) whether the *L. acidophilus* is consistently present during caries activity and absent during caries inactivity; (2) whether the reduction of refined carbohydrate intake is effective in reducing the *L. acidophilus* index; and (3) whether the reduction of a high *L. acidophilus* index is always accompanied by a reduction in dental caries frequency.

For the first objective, 1,250 individuals, between the ages of 4 and 60 years, with rampant dental caries were selected, and even though one-half of this group was 18 years or younger, a good distribution of various ages was ascertained. It was felt that such a large number of patients would provide a sufficiently dependable picture so that statistical computations could be applied. Determinations of *L. acidophilus* indices on two successive days of these 1,250 individuals confirmed beyond any doubt the findings of the Michigan school: In 87.7 per cent (1,096 cases) high *L. acidophilus* indices ranged between 1,000 and 1,000,000 colonies per cubic centimeter of saliva. Low indices up to 999 per cubic centimeter were found only in 12.3 per cent (154 cases). However, if values between 300 and 999 colonies are considered high, the total number of high indices would amount to 90.1 per cent against 8.9 per cent low indices.

The opposite picture was found in a group of 265 caries-free individuals of whom a total of 82.3 per cent (218 cases) had low indices, and 17.7 per cent

(47 cases), high indices. Even though the contrast in this group was somewhat slighter than in the rampant dental caries group, it was found in subsequent studies of these caries-free individuals that more than one-half of those with high indices developed dental caries shortly afterward. Because of these rather striking findings, it could be concluded that with few exceptions caries activity is always accompanied by high *L. acidophilus* indices, and in a state of caries inactivity these microorganisms are absent.

The second objective required a most careful and painstaking clinical study and monthly *L. acidophilus* checks. Even though this study extended over a period of five years, only a comparatively small group continued over the entire length of time because just a few cared to submit monthly samples longer than one or two years. These clinical and bacteriological studies were divided into one-year observations so that the data were comparable. Thorough clinical and roentgenographic examinations were made at the beginning and at the expiration of each year. The patients were required to record their food intake for seven days according to careful instructions. Following the first two salivary tests they were then asked to eliminate all excessive refined sugars. A detailed description of the procedure for the evaluation and correction of the food intake was published from this laboratory.[5]

Jay suggested a plan[13] to reduce the carbohydrate intake to approximately 100 Gm. daily by eliminating sugar as well as bread, potatoes, and the higher carbohydrate fruits and vegetables for two weeks during which time a rapid decrease of the *L. acidophilus* index occurred. After these two weeks he reinstated all carbohydrates with the exception of free sugar and again after two weeks the patients were permitted to include sugar in desirable amounts with one meal a day. He reported that a high percentage of patients on this regime were able to maintain a low *L. acidophilus* index, and thereby reduce caries activity. This approach has not worked too well in our hands as a practical routine procedure because many indignant mothers and even adult patients were reluctant to submit to such an extreme restriction of food intake because of fear that this regime would be a hazard to their physical well-being even if it was applied for only a few weeks.

In our study, patients were impressed with the necessity of eliminating all "excessive" refined sugars and refined flour products. Fresh fruit was to be consumed only once a day for breakfast and not during the day. The frequent use of fresh fruit for luncheon, during the afternoon hours, or after dinner before retiring caused, in many instances, the *L. acidophilus* index to remain at a high level. Among the fresh fruits, bananas were singled out because of their high carbohydrate content and stickiness. Canned, stewed, or dried fruit was also eliminated. In almost every instance, a very high proportion of calories had to be replaced by other foods with particular emphasis on proteins. Wherever there was a medical problem in existence, as for instance, allergies, endocrine disturbances, etc., the correction of food intake was made in collaboration with the family physician. Only very infrequently patients objected to such a program and expressed unwillingness to abide by such a restriction. These were primarily children who had little discipline and whose parents could

not exert their influence and authority. This elimination and substitution program when followed to the letter resulted in an immediate drastic reduction of the *L. acidophilus* index.

A total of 790 one-year observations of a random group were thus made with the following results: In approximately one-third (246 cases) the high *L. acidophilus* indices dropped to zero and remained zero throughout the year. A second third (306 cases) dropped down to zero or a very low level and either remained low or zero or fluctuated slightly. A total of 70 per cent (552 individuals), therefore, responded favorably to this program with an elimination or drastic reduction of lactobacilli following the nutritional restrictions and established this procedure as a most important tool in eliminating acid production in the oral cavity. Since nutrition problems vary with each family, personal contact with the patient was found to be most important for the success of this program. Instructions that were given by written communications or through the personnel in individual dental offices were not as effective.

The third objective was, of course, the most important one, namely, to establish whether the elimination or reduction of the oral lactobacilli would actually be accompanied by a reduction in dental caries incidence. The above 790 individual one-year observations on the behavior of the *L. acidophilus* index following nutritional correction were plotted against the development of cavities during this period. In 62.3 per cent (492 cases) no more caries was observed during one year, 17.7 per cent (140 cases) developed one or two cavities, and 20 per cent (158 cases) developed three and more cavities. Since these 790 cases were taken from a random group, it was suspected that if those patients who according to our records showed little, if any, cooperation were eliminated from these statistics, the percentage of results would be higher. By eliminating 125 uncooperative patients from the total group of 790, the results were as follows: 71.7 per cent (473 cases) did not show any new caries, 17.3 per cent (115 cases) developed between one and two cavities, and 11.6 per cent (77 cases) developed three and more cavities. Furthermore, if the reduction from ten or more cavities in the preceding year to one or two cavities during the one-year observation period was accepted as evidence of definite improvement and considered a good clinical result, the percentage of marked reduction in caries activity for the total group of 790 patients was 80 per cent. In the restricted group (665 patients) from which the noncooperative patients were eliminated, this figure rose to 88.4 per cent.

As can be readily seen, the factor of cooperation is a very important one and will influence the percentage of success in the hands of various investigators or clinicians. For this reason, it should be noted that the younger the child, the more frequently did we find a lack of cooperation, while the reverse was true for the adult group with a better understanding of the problem. In the over-all picture, cooperation was obtained by approximately 80 per cent of all patients.

Since it has been suspected by some that the actual prevention and control of decay is not due to the reduction of refined carbohydrates but rather to their substitution by other carbohydrates and proteins as mentioned this morning by Dr. Stearns, attention should be called to an investigation which Collins, Jensen,

and Becks[5] reported in 1942. In this study contrasting groups of students of the University of California of comparable age, sex, environment, and occupation were selected consisting of ninety-nine individuals who were caries inactive and never had had any caries experience and 107 individuals who were extremely caries active. Detailed dietary histories were taken by personal interviews. Even though these were evaluated against an arbitrary standard, the results of the food intake analyses were comparable. It was found that food essentials like protein, calcium, phosphorus, carotin, thiamin, ascorbic acid, and vitamin D were not more deficient in the rampant caries group than in the caries-free group, and no more adequate in the caries-free group than in the caries-free group. The caries incidence, therefore, can hardly be explained on the basis of different amounts of these essentials. The only difference with regard to food intake in these two groups consisted in the refined sugars and concentrated sweets. These averaged approximately an equivalent of ten teaspoonfuls of sugar daily for the caries-inactive group and eighteen teaspoonfuls of sugar daily for the caries-active group. Recent postwar observations in England, Italy, India, and Sweden emphasized the fact that the reduced sugar ration actually decreased the caries incidence of the population, and starvation diets during the war did not increase the caries incidence in these countries.

The above findings indicate that the fundamental work of Bunting, Jay, and co-workers could be confirmed with the following conclusions:

1. The reduction of the *L. acidophilus* index can be obtained within a period of a few weeks by reducing the excessive refined carbohydrate intake and by replacing the calories derived from these foods by increasing the intake of milk and milk products, meat, eggs, and vegetables.

2. The high degree of success establishes the reduction of refined carbohydrates at least as *one* effective means of reducing the oral *L. acidophilus* index.

3. In a group of 665 cooperative patients with rampant dental caries, the reduction or elimination of oral lactobacilli was accompanied by a decrease in caries incidence in 88.4 per cent or 588 individuals.

4. A program of dental caries control or prevention is entirely practical and effective in private practice, institutional work, and the public health service.

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DISCUSSION

DR. EASLICK.—Thank you, Dr. Becks. It is 12:30, but I think that you enjoyed that which Dr. Becks had to say. Now, before we have a very brief discussion period, I have just a few announcements to make. I yet have to get my five cents' worth in this morning. An announcement which I have to make is that the State Dental Health Directors are planning a lunch at the Michigan Union. I hope they get over there in time for lunch at the Michigan Union. And the next announcement I have to make is to refresh your minds we do have a dinner this evening. You probably will get at least $2.13 worth of food, you will listen to a speaker who is one of the local boys that made good (he got such a big name before the public that it spelled down the champion eighth grade speller in Nebraska), and you will also listen to a local boy who did not make good, who has to serve as toastmaster. Now five minutes' worth of discussion of Dr. Becks' paper. Dr. Knutson?

DR. JOHN KNUTSON (United States Public Health Service).—I just want to correct a statement, or misunderstanding of a statement as alleged to have been made. I do believe that the time is here for the dentist to charge for preventive service. I merely pointed out the dentist has not had the experience of charging for these preventive services that the medical man has had.

DR. W. A. ARMSTRONG (University of Minnesota).—I should like to ask a question for a point of information, and not in any way about discussion. Could you tell us in some quantitative terms, either in grams or teaspoons, the difference between what you regard as a satisfactory diet in refined carbohydrates and the average of such diets? What I want to get at is what quantitatively is the difference in those two types of diets?

DR. BECKS.—This question has been asked many times. Of course, I shall have to refer you back to the *individual tolerance* of sugar. We actually judge the sugar tolerance for caries safety by the number of bacteria. In other words, if you want to eat a box of candy every day and don't want to develop cavities, then it is best to check your *L. acidophilus* index first. If you should have 200,000 colonies, it would be safe on the basis of our studies to assume that with this high index, you would develop new cavities. In other words, we measure the response to sugar intake by the number of lactobacilli present. Even though a certain percentage of carbohydrates is required for physical well-being, they don't have to come from refined sugars. Does that answer your question?

DR. ARMSTRONG.—No, I am still not satisfied. I think you might give some indication as to how low these refined carbohydrates go in one average case—50 Gm., 10 Gm., zero, or 100 Gm., or what?

DR. BECKS.—That is for caries prevention, you mean? Well, I don't believe that we can speak in terms of an "average case," because the response to sugar intake varies with each individual. Perhaps Phil Jay has a better answer.

DR. PHILIP JAY (University of Michigan).—No. I don't think we have the information to answer that as yet. We arrived at our rather peculiar method of restricting the sugar; we restrict the sugar to a much lower level than Becks does, and then after a period of time we allow them to have sugar at one meal a day, and you will find that after the preliminary period, 70 per cent of the individuals who have had rampant caries will tolerate, I should judge, about 50 Gm. at least of sugar a day and not have caries activity. Does that answer your question somewhat?

DR. ARMSTRONG.—That comes much closer than Dr. Becks did. The point I am trying to make is that if 50 Gm. is a low carbohydrate intake what is high—200, 300, 500 grams?

DR. JAY.—Yes, yes.

DR. ARMSTRONG.—Well, 50 is 10 per cent of 500. It still seems to me that 50 Gm. of carbohydrate is an awfully lot of carbohydrate for the oral flora. That much should furnish sufficient substrate. I believe your work and I understand it, but how can you explain the mechanism by which this much carbohydrate reduction reduces the bacterial flora? Within the test tube, these concentrations would certainly be ample for bacterial growth.

DR. BECKS.—Well, as I said before, we judge caries activity and sugar tolerance (which is not a very good term) entirely by the number of lactobacilli because we don't have a qualitative difference betewen the various sugars and various sweets. For instance, we don't know the difference between brown sugar and white sugar in terms of their effect on caries activity or their effects on lactobacilli. The same goes for fruit sugar and other refined sugars. We go entirely by the number of lactobacilli. If they drop out, we keep our patients on this regime for three months. Then we add more fresh fruit to the daily food intake, and if the lactobacilli don't return, we allow a dessert every second or third day. It has been our experience that after three months the majority of individuals can tolerate larger amounts of sugar again without increasing their *L. acidophilus* index again.

DR. EASLICK.—Dr. Bibby, I think had his hand up.

DR. BASIL G. BIBBY (Eastman Dental Dispensary).—I have been rather impressed, in reviewing the literature over a number of years, with the association between the frequency of eating and dental caries. In fact, on the other side of the almost preventable cure, I seriously have been advocating reduction in the frequency of eating as a caries preventable control. I should like to know if either Dr. Jay or Dr. Becks has noticed any influence, any relation between the frequency of eating in addition to the carbohydrate content on the life or activity of the lactobacillus?

DR. EASLICK.—Dr. Bibby, when you state a question like that you start a fight.

DR. BIBBY.—Oh, I don't think so; there was no fight intended.

DR. EASLICK.—I mean I don't know which one to ask first. I shall take a chance, since Dr. Jay is local, and ask Dr. Becks.

DR. BECKS.—Well, there might be a correlation, Dr. Bibby. There is apparently a difference whether you eat half a box of candy at one time or throughout the day. We try to have our patients avoid eating, for instance, fresh fruit between meals or again after

dinner, or drinking milk after dinner before retiring, because we have the impression that the frequency of eating has something to do with the development of the lactobacilli. During the fresh fruit season our patients are to cut out the fresh fruit during the day—they can have fresh fruit in the morning for breakfast only. "Chronic apple eaters" and youngsters who consume large numbers of oranges when they come out of school in the afternoon, usually have a very high *L. acidophilus* index. This frequent eating of fruit sugars might have a definite relation to caries incidence. However, I don't think that we have sufficient evidence of a causative relationship as yet.

DR. EASLICK.—Dr. Jay, are you satisfied with Dr. Becks' answer?

DR. JAY.—Oh, yes.

DR. EASLICK.—Dr. Robinson?

DR. HAMILTON B. G. ROBINSON (Ohio State University).—Dr. Becks made a statement and I think it might be taken with wrong intent although I know what he intended. He has intimated that adequacy of diet is unimportant. Now, when Dr. Becks says that, or when any of us say that, we are speaking only in respect to dental caries. The adequacy of the diet isn't going to affect the dental process. Now, some nutritionists might suggest that we are advocating that people don't need an adequate diet at all. They still need an adequate diet, but from the general standpoint. As far as dental decay is concerned, the adequacy of the diet does not seem to be very important. Although it might be from other oral standpoints, such as affecting the periodontal or gingival structures. Perhaps dentists should continue to advocate an adequate diet, but not to have the concept that it is going to stop decay. That is a very good way to produce a lot of harm. You say to a child, "You do this, and do that, and drink this and drink that and eat this food and you won't have any more decay." Poor little Johnny comes back in six months, and you reach over and pull down the handpiece and work on Johnny's teeth and Johnny loses his whole faith in you. You can't specifically give an additive dietary for the teeth, but don't let nutritionists interpret that as saying we don't believe in adequate diets. I think we all believe in adequate diets, but some of us don't believe that it is very important in the caries process.

DR. EASLICK.—I think I shall exercise a chairman's prerogative and declare this session adjourned.

DR. EASLICK.—We shall begin right where we left off at 5 o'clock this afternoon, with some more dental caries control. The first day that you were here, I promised you, as a coming extracurricular attraction, the man who caused the Nebraska champion, eighth grade speller to be disqualified in a national spelling bee (he could not spell the word, Lactobacillus acidophilus), and the holder of the preschool long-distance spitting record. When they were but three years of age he examined saliva samples from the five Dionne quintuplets all the way from Colander, Ontario. Tonight his subject is different. It is "The Present Status of Caries Control Measures—A Workshop Summary." I think that every one of you should know that it is a pleasure to turn over this part of the program to Dr. Philip Jay.

THE PRESENT STATUS OF CARIES CONTROL MEASURES*

PHILIP JAY, ASSOCIATE PROFESSOR OF DENTISTRY, UNIVERSITY OF MICHIGAN, ANN ARBOR

It is my assignment to summarize the vast amount of material which has been presented at this Workshop. The task is an easy one, if not actually superfluous. I have been familiar with the studies of all the essayists as I have listened to most of them discuss their problems many times, but I am compelled to remark, at the risk of breaking the long-established taboo against dental caries research workers complimenting each other, that they certainly carried the mail this past week.

All of the speakers appreciate the rigid requirements for effective research as stipulated by Dr. Wallace at the opening of this meeting. You have listened to the various phases of dental caries research presented dispassionately and very simply by lecturers whose observations were supported by data which were presented to you for inspection. Your conclusions may differ, even as there are differences of opinion among the investigators who have gathered these data. That is quite natural since all of us view this material from vantage points based upon backgrounds of varied personal experiences.

This conference, however, was not characterized by confusion. After listening to and participating in these discussions those of you whose job it is to disseminate dental health material should have little patience with the oft-repeated phrase, "Little is known about the etiology and control of dental caries." A great deal is known about both. The mechanism by which the carious lesion is produced has been clarified by studies which were described during this Conference. It is perfectly obvious, from the data which have been presented, that the onset of caries activity can be predicted and that the development of the lesion can be prevented. That, it seems to me, is knowing a great deal about a disease. It is true that the known control measures are not practical in every case, but the fact that they are workable at all, even under very special conditions, is evidence that dental caries research has been fruitful and that the future is not as bleak as it appeared not so many years ago.

In order to appreciate fully the significance of this Conference it is well to remind you that the lecturers were not selected because of any uniformity of opinion. Nevertheless, it was apparent that there was agreement on some points. That should be a comfort to those of you whose job it is to transfer information from the laboratory to the field.

Writers on this subject have shown a tendency of late to refer to dental caries research workers as constitutionalists or environmentalists, depending upon whether they think that the disease is influenced by systemic or environmental factors. We in Ann Arbor, for instance, have been cited as horrible examples of the environmentalists because so much of our attention has been focused upon the relation of lactobacilli to dental caries activity, and also because we were never able to relate caries to the state of nutrition. As a matter of fact, it would be ridiculous to assume that conditions surrounding the teeth, which

*Presented at the dinner of The Caries Control Conference on Friday evening, Sept. 12, 1947.

exist in a body cavity, are not influenced by systemic conditions. Consequently, it is of interest to determine which systemic conditions might regulate such dental environmental factors as the oral flora and conditions which permit the production of acid on tooth surfaces.

We have heard a lot about both during this Conference. All of the speakers agreed that caries control measures are effective through their influence upon the tooth's environment. It was also apparent from these discussions that acid production in the mouth is of major importance.

From Dr. Chase's scholarly dissertation, it is evident that the dental tissues are vulnerable to the action of acid. Dr. Stephan demonstrated differences in the acid potential of dental plaques. He pointed out that the pH of the plaque drops immediately after the ingestion of sugar and that the extent of the drop varies directly with the degree of caries activity on each particular tooth surface. From this it may be assumed that the nature of the bacteriological flora of the dental plaques may vary from tooth to tooth. This should suffice to relieve anyone of the delusion that the caries process is a simple one. However, two facts are definitely known: a quantitative relationship must exist between acid production and the carbohydrate substrate from which the acid is produced. It has also been shown that the enzyme system which is responsible for the acid production on the tooth is bacterial in origin. Inasmuch as the flora associated with caries is characterized by the presence of lactobacilli, most attention is being centered upon the fermentation of carbohydrate in the mouth and upon the growth characteristics of lactobacilli.

A relationship has been shown to exist between the presence of carbohydrate in the mouth and the growth of lactobacilli. The carbohydrate then may have a double-barreled action in that it provides both the substrate for acid production and a growth factor for the bacterial which brings about the acid production. Dr. Becks has described the clinical application of this knowledge. By restricting the amount of sugar in the diets of a large number of patients, he has observed a drop in the number of oral lactobacilli and a reduction in caries activity. While he was describing the diet which he employed so successfully in California I could catch the gleam in Genevieve Stearns' shining eyes because it was apparent that the Becks' diet is nutritionally adequate and in good balance and certainly Dr. Stearns and her associates in Iowa have for years maintained that an adequate diet which meets the nutritional requirements of the body will serve to prevent dental caries as well. We have been impressed with the evidence presented by Dr. Stearns relative to the importance of nutrition in the maintenance of good growth and health. Many of us are wondering, though, if caries wouldn't have been arrested in the Iowa studies on diets which were less perfect nutritionally but which contained the same low level of sugar intake. We think we made just that observation here at Michigan when we demonstrated an extremely low incidence of dental caries in a group of children on a low-sugar diet which was not optimum in a nutritional sense. Although I can't imagine Dr. Stearns ever experimenting with children on inadequate diets, I have an idea that some day the Iowa and Michigan studies will come out at the same place.

Dr. Robinson was well supplied with the ammunition necessary to demonstrate how completely dental caries is disassociated from general disease as well as with perfectly normal states of which pregnancy is a classic example. Certainly Dr. Armstrong presented sufficient evidence for an understanding of why dental caries is not associated with disturbances of mineral metabolism.

There are dentists in this room who have observed patients free of dental caries even though they consume large amounts of sugar. They haven't seen many such patients it is true, but they exist, nevertheless. That is why Dr. Williams is interested in the serological characteristics of lactobacilli. There are times when conditions in the mouth are not compatible with the growth requirements of lactobacilli so that the immunological implications should be explored. He has found, as have others, that lactobacillus agglutinins may be produced in the blood of human beings. He has also learned that before much more can be learned about immunization against lactobacilli, it will be necessary to untangle a complex serological relationship which exists between many types of lactobacilli. It is also apparent that Dr. Williams is well on the way to clarifying this difficult problem.

There are other possibilities in the caries prevention field. The role of silver nitrate in caries control has been discussed for years. Among us here, Olin Hoffman of Iowa thinks it works and John Knutson of Washington thinks it doesn't. Dr. Zander, whose job it was to render a verdict, "isn't saying." He is of the opinion that silver nitrate could be given a fairer trial. If I have misconstrued his deductions, I'm sure he'll let me know about it.

Dr. Hine was unable to tell us that prophylaxis and tooth brushing are very effective means of combatting dental caries. His assignment was most difficult since practically no statistical data are available on this subject. I think we can deduce from Dr. Hine's presentation and the discussion which followed it that the prevalence of dental caries will not be reduced by tooth brushing but that the lesions themselves are less extensive when they occur on teeth which are regularly brushed.

Remarkable results are not to be expected from tooth brushing unless of course you brush your teeth with Dr. Kesel's tooth powder. He has found that lactobacillus counts are reduced following the use of a tooth powder which contains dibasic ammonium phosphate and urea. He has gathered sufficient data to theorize that the ammonia ion inhibits the lactobacillus, thus allowing the entrenchment of *Bacterium aerogenes*. This organism produces an enzyme which deaminizes the amino acids, thus liberating ammonia making it difficult for the lactobacillus to re-establish itself. A cycle is thus created which to the lactobacillus is "vicious." Dr. Kesel and his associates are conducting a study in several Illinois cities in which school children use the preparation under strict supervision. The outcome of this extensive program will be awaited with great interest. Certainly I should prefer a tooth brushing regimen to one which requires the diligent selection of low-carbohydrate foods.

In a sense, antibiotics play an important part in the inhibition of lactobacilli through the use of the ammonium urea compounds by favoring the growth of

B. aerogenes. According to Dr. Hill's studies, lactobacillus counts can be reduced by the use of penicillin dentifrice. Neither Dr. Kesel nor Dr. Hill has presented clinical data which indicate the extent to which caries is controlled by these means, but the bacteriological evidence is good. Some allergists frown upon the regular use of penicillin, and there is the problem of maintaining the potency of penicillin at room temperature. To date, Dr. Hill has seen no contraindication for its use because of sensitization. The fact that he is not advocating the use of this drug for the control of caries at this time does not minimize the importance of these studies. The discovery of a means of altering the oral flora contributes enormously to the knowledge of the etiology and control of this disease.

One of Dr. Hill's famous protégés was fortunate to make his debut in a problem about which there has been no controversy. I am referring, of course, to Dr. Arnold and his interest in the epidemiology of caries with reference to fluorine in domestic water supplies. Everyone agrees that low caries attack rates occur in populations supplied by waters which contain fluorine. One reason for the lack of argument about this fact is due largely to the degree of perfection of the epidemiological techniques employed by Trendley Dean, Arnold, and their associates. They had incontrovertible evidence before they made known their findings. You have noticed that Dr. Arnold is unwilling to state that the addition of sodium fluoride to water will reduce dental caries activity. I have enjoyed witnessing several episodes during which some pretty insistent inquisitors have attempted to extract such a positive statment from him. Of course I've had similar experiences myself, but it's much more fun watching Arnold's reaction to these traumata. The fact remains that it is not yet known whether or not the addition of fluoride will reproduce the conditions which we have observed in areas where fluorine occurs naturally. Our a priori reasoning tells us that positive results will be obtained in experiments now in progress, but it certainly is dangerous for research workers to draw conclusions from data which have not yet been obtained. Even if we should find that water treatment is effective, it is apparent from Dr. Arnold's review of this subject that calcium fluoride and bone meal in the products now on the market are not effective caries control agents. At any rate, it is not conceivable how fluorine taken internally would affect the susceptibility of teeth already calcified. Dr. Knutson has presented very impressive evidence which indicates that sodium fluoride applied topically to teeth reduces the incidence of caries. His studies confirm the observations of others that multiple applications of fluoride to the teeth of children up to 15 years of age are effective. When Arnold, Dean, and Singleton applied sodium fluoride to the teeth of 19-year-old men, no beneficial results were observed. Their study differed from those yielding positive results in that they treated an older age group and, although they made three applications, the treatments were given at the same sitting. This would indicate that either the age of the patients or the interval of time which elapses between the applications is very important. Dr. Knutson's findings indicate that four treatments given within a two- or three-week period produce optimum results in children up to fifteen years of age. It is possible that four treatments applied to adults during the same time inter-

val would be equally effective. At present, there is no statistical evidence indicating whether or not adults may benefit by this treatment. It is interesting to speculate upon the mode of action of fluorine. Several possibilities have been considered. Fluorine is an enzyme poison and could interfere with the fermentation of sugars into acid. It could reduce acid activity by lowering the solubility of the teeth, or by inhibiting the growth of the bacteria themselves. Perhaps it works in all three ways. In this connection it is a curious fact that when applied topically fluorine does not reduce the oral lactobacillus count despite the fact that lactobacillus counts are unusually low in individuals whose teeth were calcified in fluoride areas. It is quite obvious that we have here a fascinating problem. Fosdick will very likely come up with the answer some day.

His studies have thrown a great deal of light on the mechanism by which acid is produced on the teeth from carbohydrate. Dr. Fosdick has experimented with enzyme inhibitors in the hope of finding a method of treating sugar so that it may be used with impunity. Two-methyl-1, 4-naphthoquinone is such a substance. He has presented evidence which indicates that the use of chewing gum containing it has a caries-inhibiting effect. Although it is theoretically sound, I do not think that the practicability of this procedure has been established. I have the distinct feeling that Dr. Fosdick will find a way, but I hope it won't require the regular use of chewing gum.

At any rate, Bibby has shown that chewing gum isn't a very good vehicle for fluorine. He was one of the first to obtain satisfactory results with sodium fluoride used topically but has not been able to find that mouthwashes or other salts of fluorine are practical.

In closing these rather rambling remarks, I hope that the workers in public health who have attended this Conference agree with me that they can go home with some definitely established facts about dental caries control. First of all, dental caries activity can be greatly reduced by the adoption of low-sugar diets. That these diets should meet all of the nutritional requirements for good health is just plain common sense. It is also clearly evident that it is possible to maintain caries-free teeth in a poorly nourished body. That, of course, is merely of academic importance.

Dental caries activity can be reduced by the topical application of sodium fluoride. Mineral and fluoride preparations now being marketed are not effective. Of the measures in the immediate offing, I am most encouraged by the possibilities of fluorination of public water supplies, the use of dentifrices utilizing the principle of dibasic ammonium phosphate and urea, and the treatment of fermentable sugars with enzyme-inhibiting substances. It has been a long, hot week, but I hope you have benefited by these discussions as much as I have.

DR. EASLICK.—You are well fed, relaxed, and ready for more. I know that the next speaker is relaxed because he doesn't know that he is the next speaker. You may be sure that none of these proceedings have escaped his attention. Dean Bunting, will you please tell us what you are thinking about?

DR. BUNTING.—I am very pleased to have the opportunity of expressing my personal appreciation of this significant conference on dental caries. As I have sat through the sessions of the week and have listened to the presentations made, I have been impressed with the scope and the quality of study that has been devoted to this problem. It recalls to me the early days of dental caries research and by comparison shows so clearly how much progress has been made.

It has been my good fortune to live through and to be intimately associated with a long period of evolution in the study of dental caries. I was privileged to know W. D. Miller personally and, had he lived, I was to have been his assistant in the new laboratories which were built for him in the new dental building here. That was in 1907, just forty years ago.

Following his untimely death, I was left alone to carry on in this school the investigations in dental caries and pathology to which Miller had contributed so largely. For this, I was wholly unprepared and it was many years before any substantial progress was made. In these early years of development, I was associated with Faith Hadley, U. G. Rickert, and Mary Crowley. Our progress was slow and halting until in 1930 when we obtained a substantial grant from the Children's Fund of Michigan to undertake a concentrated and coordinated study of the problem in which trained workers in the fields of bacteriology, nutrition, chemistry, and dentistry were employed. In the organization of this Michigan Dental Caries Research Group and the conduct of the studies, Dr. Philip Jay has always played a very important role.

If, as the result of our studies here, we have been able to make some significant contribution to the knowledge of this subject, we are indeed very glad. Since the termination of our group studies, many new developments have been brought forward, and previous observations have been given greater substantiation. In this school Dr. Jay has carried on notable studies of the carbohydrate factors and, in conjunction with the National Institute of Health, has taken an active part in the experimental fluorination of community water supplies. Major research projects are also being conducted in other centers and the investigators are delving into many new and untilled fields of study related to the problem of dental caries. It is with great satisfaction that we view these advancements in knowledge and the gradual extension of the frontiers of understanding of this intricate problem.

Looking back in reminiscence over the years of active study of dental caries, we have seen many alluring theories come and go; panaceas and methods of prevention have been proposed and widely accepted, only to be found that they were but will-o'-the-wisps that led nowhere. Today they are gone and forgotten, except for their record in the annals of our profession, but one day they were broadcasted throughout the land as great discoveries which would forever abolish the scourge of dental caries. I have often thought, in reviewing the stages by which our present knowledge has been evolved, that if I had the pen of a Paul de Kruif, I would write a book outlining the interesting trends of thought through which we have passed and I would call it "The Romance of Dental Caries." To me it has been a glamorous quest that has been at times almost an obsession. Now that I can see so much substantial progress as has been reported at this conference and the zeal and true scientific approach with which the investigators are attacking the problem, I have real hope that even in my lifetime, this great question may be solved, and that the boon of freedom from dental caries may be vouchsafed to mankind through dental research.

May I wish you Godspeed.

REPORTS OF EVALUATING COMMITTEES*

The Mechanism of the Caries Process

COMMITTEE I, whose assignment it was to study and report on the mechanism of the caries process, agreed that before attacking the problem of the mechanism of the caries process an acceptable definition of dental caries should be formulated. After the formulation of the definition it was agreed to consider in chronologic order the various factors concerned in the mechanism of the caries process.

In the preparation of the final report the committee agreed to follow the policy of basing its conclusions on the scientific evidence which has been substantiated by acceptable procedure. However, it also was agreed to include some information which may be an important factor in the mechanism of the caries process but to state that additional evidence is needed to substantiate the opinions based on such information.

DEFINITION

Dental caries is a disease of the calcified tissues of the teeth. It is caused by acids resulting from the action of micro-organisms on carbohydrates, is characterized by a decalcification of the inorganic portion and is accompanied or followed by a disintegration of the organic substance of the tooth. The lesions of the disease predominantly occur in particular regions of the tooth, and their type is determined by the morphologic nature of the tissue in which they appear.

FORMATION OF ACID

The acids involved in the caries process are derived from carbohydrate substances after they have been acted on by microbial enzymes.[1-16] The enzymes have their origin from the microbial flora which has an enzyme system capable of breaking down carbohydrates to acids. Any micro-organism or combination of micro-organisms which is capable of maintaining an acid potential sufficient to decalcify enamel is capable of initiating dental decay.

MICRO-ORGANISM

The following micro-organisms have been found capable of producing this acid potential *in vitro*:[12] lactobacilli, aciduric streptococci, diphtheroids, yeasts, staphylococci and certain strains of sarcina.

The lactobacilli are the only micro-organisms which have been found to show a significant increase in incidence with the initiation of caries.[17-20] Lactic acid has been demonstrated to be present in the caries lesion by a specific method.[5, 21] Phosphoglyceric, pyruvic, formic, acetic, propionic and butyric acids have been demonstrated to be formed by microbial action in saliva-sugar mixtures.[22]

DECALCIFICATION

Whether or not the acid formed will decalcify the enamel of a tooth is dependent on the degree of acidity and the time that acid is in contact with the tooth. There is good evidence to indicate that an acid potential in the vicinity

*Reprinted, with additions, from *J. A. D. A.* 36: 3, 1948.

of pH 5.0 is sufficient to decalcify the inorganic structure of a tooth.[23, 24] The time that the acid must be in contact with the tooth in order to produce decalcification is not known.

SUBSTRATE AND ENZYME SYSTEM

A suitable substrate and enzyme system are necessary for the production of acid.[1-16] The preponderance of evidence indicates that the acids are formed by that which now is accepted as the "anaerobic phase" of carbohydrate degradation.[1-16] It is recognized that other paths of degradation are possible, but as yet they have not been demonstrated to occur in the mouth. The substrate is derived from ingested carbohydrate or carbohydrate from any other possible source. The enzyme systems are known to be derived from the micro-organisms, but they also may be derived from the oral tissues and secretions.

RETENTION OR DISSIPATION OF ACID

The effect of the caries process on the tooth is made possible by circumstances or structures which retain sufficient acid in contact with the tooth substance. These factors, not necessarily listed in their order of relative importance, include (1) the dental plaque, an organic nitrogenous mass containing multitudes of micro-organisms firmly adhering to the teeth,[12, 25-28] (2) the anatomic characteristics of the tooth, (3) the position or arrangement of the teeth in the dental arch and (4) the presence of dental appliances.

INHIBITORY FACTORS

There are also natural factors in the mouth which contribute to the dissipation of acids formed on the tooth surface, such as the amount of saliva and the buffering capacity of the saliva.[29-33] A few reports have suggested that resistance to dental caries in human beings may be accounted for by inhibitory substances.[17, 34-43] Until such a time as more evidence is available to indicate the identification and action of these factors it is necessary merely to recognize their possible role.

The factor which determines whether or not the caries lesion develops depends on the interrelation of all the preceding variables, so that no single factor may be construed as the general cause of all dental caries.

Once the process as described is initiated, the morphologic characteristics of the lesion are determined by the effect of this process on (1) the anatomic structure and the substances of the tooth, (2) the extent, intensity and duration of the attack and (3) the reaction of the tissues of the tooth. Two common sites of initial lesions are intact enamel and pits and fissures or defects of the tooth.

The first visible evidence of caries on an intact surface of enamel is a change in the optical properties of the enamel. By reflected light this change appears as a whitening, and by transmitted light it is noted as a darkening of the region. Microscopically the rods appear more definite, and the cross striations and incremental lines become more prominent. The substance of the enamel at the surface may be noted to disintegrate, and micro-organisms and debris resulting from the loss of substance may be observed in the interspaces. Pigmentation of the lesion may or may not be noted.[44]

When the process reaches the dentino-enamel junction, expansion of the lesion in this region takes place. Then it progresses through the dentinal tubules toward the dental pulp. At this stage of the process there may be undermining of the overlying enamel. Micro-organisms may precede the process of decalcification of the organic matrix. Small isolated regions of matrix destruction may be observed about some of these microbic masses in the tubules, and these regions may be seen to coalesce. Accompanying this process there is a progressive loss of substance.[6, 44]

Defects in the enamel such as lamellae, certain pits and fissures, and artificially produced defects may afford pathways for the entrance of micro-organisms and may result in caries proceeding from the surface of the defect.[44, 45]

Caries also may begin on the thin layer of cementum and proceed into the dentin.[6, 44]

Respectfully submitted: George W. Teuscher, D.D.S., chairman; Ralph E. McDonald, D.D.S., secretary; Paul W. Clopper, D.D.S., Michael A. Cox, M.D., Allen O. Gruebbel, D.D.S., Olin E Hoffmann, DD.S., Hugh R. McLaren, D.D.S., Harold J. Noyes, D.D.S., M.D., Dorothy Permar, M.S., Katharine A. Polevitsky, M.D., and Fred Wertheimer, D.D.S.; Basil G Bibby, DD.S., Samuel W. Chase, Ph.D., Mary C. Crowley, M.S.P.H, Robert M Stephan, D.D.S., Mabel I. Tramp, M.P.H., Ned B. Williams, D.D.S., Helmut A. Zander, D.D.S., and Leonard S. Fosdick, Ph.D., consultants.

Note: No changes in the original statement resulted from the discussion period. It was moved by Dr. Owens that the report be accepted. The motion was seconded by Dr. Gruebbel and was carried unanimously.

Relation of Systemic Conditions to Caries Process

For the sake of brevity and ease of discussion, the members of Committee II decided to prepare an analysis of the findings and conclusions, with a table showing the approach to the etiologic factors of the caries process.[46-54]

The systemic conditions were divided into seven categories; namely, (1) pregnancy and lactation, (2) general health and nutritional status, (3) diabetes, (4) endocrine glands, (5) infectious diseases, (6) emotional state and (7) constitution and heredity. Each subject was assigned to a subcommittee for a review of the available literature. The committee's discussion periods were devoted to a critical appraisal of individual committee reports. The pattern used in the evaluation of the published material follows:

ETIOLOGIC FACTORS

I. Indirect factors influencing caries
   A. Tooth
      1. Composition
      2. Morphologic characteristics
      3. Position
   B. Saliva
      1. Composition
         a. Organic
         b. Inorganic

2. pH
3. Quantity
4. Viscosity
5. Antibacterial factors

C. Diet
1. Physical factors
Quality of diet
2. Local factors
a. Carbohydrate content
b. Vitamin content
c. Fluorine intake

II. Direct causes of caries
A. Acidogenesis
Carbohydrate degradation to acid
B. Proteolysis

PREGNANCY AND LACTATION

Pregnancy and lactation in themselves are not a cause of dental caries. If pregnancy increases the susceptibility or resistance to dental caries, it can do so only by changing the environment of the tooth. Clinical studies indicate that pregnant women do not have a higher rate of caries than do comparable non-pregnant women.

GENERAL HEALTH AND NUTRITION

A careful review of scientific reports reveals that sickness, general health or nutritional status has no significant bearing on the caries process. In fact, several scientific reports reveal that malnourished persons have a decreased incidence of dental caries. Sickness may have a definite influence on the composition, quantity, viscosity and bacterial or antibacterial factors of the saliva, but there is no acceptable evidence to show the influence that these factors may have on the caries process.

DIABETES

It has been stated that prior to the insulin era tooth decay was rampant in persons with diabetes. These reports attempted to link the diabetic state with tooth decay, with special stress on cervical caries. The committee would like to point out that there have been no modern adequate studies of diabetic children who were not under treatment and that any differences which might be noted were the result of treatment rather than of the disease. All studies seemed to indicate that there was a need for a comparative study of young diabetic patients under various degrees of control and uncontrol.

ENDOCRINE GLANDS

Endocrine glands occasionally are stated to be involved directly in inducing normal or abnormal tooth structure, in determining the position of the teeth in the arch or in influencing salivary secretions and, through such relationship, have an effect on the resistance or susceptibility to caries. The allusions to these

FACTORS TO BE CONSIDERED IN THE RELATION OF SYSTEMIC CONDITIONS TO THE CARIES PROCESS

ETIOLOGIC FACTORS	PREGNANCY AND LACTATION	GENERAL HEALTH AND NUTRITION	DIABETES	ENDOCRINE GLANDS	INFECTIOUS DISEASES	EMOTIONAL STATE	CONSTITUTION AND HEREDITY
	I. INDIRECT FACTORS INFLUENCING CARIES						
A. Tooth 1. Composition 2. Morphologic characteristics 3. Position	1. None[46-48]	1, 2, 3 During development only[46-48]	1. None 2. None[46-48] 3. None	1, 2, 3 During development only[46-48, 54]	1, 2, 3 During development only[46-48]	1. Unknown 2. Unknown 3. Unknown	1, 2, 3 During development only[46-48]
B. Saliva 1. Composition 2. pH 3. Quantity 4. Viscosity 5. Antibacterial factors	1. Unknown 2. None[49, 50] 3. Unknown 4. Unknown 5. Unknown	1. Unknown 2. Unknown 3. Unknown 4. Unknown	1. Unknown 2. Unknown 3. Decreased[51] 4. Unknown 5. Unknown	1. Unknown 2. Unknown 3. Unknown 4. Unknown 5. Unknown	1, 2, 3, 4, 5 Variable or unknown[51]	1. Unknown 2. Unknown 3. Unknown 4. Unknown 5. Unknown	1. Unknown 2. Unknown 3. Unknown 4. Unknown 5. Unknown
C. Diet 1. Quality 2. Carbohydrate content 3. Vitamin content 4. Fluorine intake	1, 2, 3, 4 Same as in nonpregnant women	1, 2, 3, 4 Same as in normal persons	1, 2, 3, 4 Same as in normal persons except where carbohydrate content is reduced[52-53]	1, 2, 3, 4 Same as in normal persons	1, 2, 3 Same as in normal persons	1. Unknown 2. Unknown 3. Unknown 4. Unknown	1, 2, 3 Same as in normal persons
	II. DIRECT CAUSES OF CARIES						
A. Carbohydrate degradation to acid (acidogenesis)	Same as in nonpregnant women	Same as in normal persons	Same as in normal persons	Same as in normal persons	Same as in normal persons	Same as in normal persons	Same as in normal persons
B. Proteolysis	Same as in nonpregnant women	Same as in normal persons	Same as in normal persons	Same as in normal persons	Same as in normal persons	Same as in normal persons	Same as in normal persons

influences are notably general, often are indefinite and frequently are based on assumptions that conditions which modify bone also modify teeth. The committee found no published significant proof or clinical evidence of the relation of endocrine disturbances and the caries index.

INFECTIOUS DISEASES

Most of the literature on the incidence and progress of caries in patients who are suffering from infectious disease would seem to indicate that the systemic condition has little or no effect on dental caries. Before the indirect effect of systemic disease can be established fully, the relation between hypoplasia of the enamel and caries should be established. However, it is felt by the committee that further research in this aspect of the problem certainly is indicated.

EMOTIONAL STATE

Since there is no clinical or experimental evidence to support the theory that the emotional state may influence dental caries, the committee felt that any further reports should be supported by clinical evidence.

CONSTITUTION AND HEREDITY

Although a genetic factor related to caries resistance has been established in laboratory animals, only familial relations have been demonstrated for man. Further study is indicated.

Respectfully submitted: Ruth Martin, D.D.S., chairman; Joseph Cabot, D.D.S., secretary; Harry W. Bruce, Jr., D.D.S., Clifton O. Dummett, D.D.S., Jack M. Earle, D.D.S., Laverne H. Jacob, D.D.S., Edward O. Minniear, D.D.S., Ruth F. Rogers, A.B., Laren W. Teutsch, D.D.S., and Kathryn L. Whyte, D.D.S., Mildred Harter, A.B., Donald A. Kerr, D.D.S., and Hamilton B. G. Robinson, D.D.S., consultants.

Note: Two minor changes were made in the original statement. Dr. Teuscher moved the adoption of the report. Dr. Strusser seconded this motion, and the motion was approved unanimously.

Prophylaxis, Toothbrushing, Chewing Gum, Dentifrices and Mouthwashes

Some of the questions submitted to Committee III have been revised and are stated in their revised form in this report. A few of the submitted questions have been deleted, and a few others have been added.

PROPHYLAXIS

Definition: Dental prophylaxis is a process which consists of (1) removal of all foreign material from the clinical crowns of the teeth (all surfaces above the gingival attachment), (2) smoothing and polishing the tooth surfaces by means of scalers, rubber cups and brushes, the porte polisher, dental tape and fine cuttlefish strips when needed and (3) polishing and correction of improper margins of restorations.

The answers which follow are based on the first two portions of this definition. The literature contains surprisingly little scientific data on prophylaxis

as related to dental caries, and, therefore, definite conclusions are not possible at this time.

Question No. 1: Will a prophylaxis remove the bacterial plaques from the teeth?

The literature indicates that thorough prophylaxis will remove bacterial plaques from the accessible surfaces of the teeth but not from the inaccessible surfaces, such as pits, fissures and contact points. The evidence presented is in the form of clinical observations and is not expressed in data which may be subjected to statistical analysis.

Question No. 2: Will a careful prophylaxis performed three times per year reduce the dental caries attack rate?

There is no statistical evidence that prophylaxis three or even more times a year will reduce the dental caries attack rate.

Question No. 3: How frequently should a careful prophylaxis be given to prevent plaque formation? Cavity formation?

Since there is no statistical evidence that prophylaxis will reduce the caries rate, it is impossible to state how frequently it must be provided to prevent cavity formation. Evidence has been presented that plaques will grow back over tooth surfaces in one to four days,[55] which indicates that it is impractical to perform a prophylaxis frequently enough to prevent plaque formation.

Question No. 4: Can the tooth be damaged by prophylaxis?

There is no evidence that dental prophylaxis as usually given at intervals of several months is harmful to the teeth.

TOOTHBRUSHING

Question No. 1: What technics may be accepted as satisfactory for brushing the teeth?

The acceptable evidence does not show that one technic of toothbrushing is more effective than another for removing foreign material from tooth surfaces. On the basis of clinical observations, many dentists prefer the Charters' vibratory technic, because of its supposed superior polishing of interproximal surfaces. This method is difficult to learn and probably is not performed well by children or by the adults who will not spend the necessary time.

Since no single procedure has proved to be ideal, selection should be made on the basis of the needs of each patient. Toothbrushing can be more pleasant and effective with an acceptable dentifrice. Few studies have been made of the value of the toothbrush alone.

The following technic has been used for many years and is suggested by some as adequate for the average young child. A vibratory method (Charters') can be taught to the child later in life.

The brush should have a straight handle, with a small head (perhaps 1¼ by ⅜ inches) with two rows of bristles, each row having four or five tufts carefully trimmed to the same height. The brush should be small enough to be handled with ease; children should be given smaller brushes than adults.

The teeth should be brushed in a definite order, to be certain that all areas are covered. The occlusal surfaces are brushed first, a sweeping motion forward

and backward being used, first in the upper and then in the lower jaw. The other surfaces are brushed with a rolling motion. The sides of the bristles are placed against the gingival tissue in the upper left molar region, and the teeth are brushed downward (occlusally), slight pressure being used when the bristles touch the buccal surfaces of the teeth. Three to six strokes are given to each area in the following manner: (1) brush the upper left molar region; (2) move the brush anteriorly and brush the buccal surfaces of the teeth around to the right molar region; (3) brush the palatal surfaces of the upper teeth, starting on the right side and continuing around to the left side; (4) brush the lower teeth from the gingival tissue upward (occlusally) beginning with the lower left molars and continuing anteriorly and around to the lower right molar region; (5) brush the lingual surfaces similarly in like sequence from right to left.

Question No. 2: Will these technics harm the teeth?

There is no scientific evidence available that these technics, correctly applied, will harm the teeth or cause gingival recession.

Question No. 3: How often should the teeth be brushed?

There is evidence that teeth should be brushed immediately after each ingestion of food.

Question No. 4: For how long a period at each brushing?

No definite length of time has been established as ideal for toothbrushing, because the time will vary from patient to patient. Probably the average young adult should brush each area six to eight times, and children should brush each area three to six times.

Question No. 5: When should the teeth be brushed?

Answered under No. 3.

Questions No. 6 and 7: These questions were consolidated and changed to read: Will brushing immediately after the ingestion of sugar reduce the amount of acid formed?

There is evidence in the literature that toothbrushing under these circumstances will reduce the total amount of acid produced. It has been stated that toothbrushing will remove most of the accessible plaque material, leaving so little that a satisfactory measurement of pH change cannot be obtained.[55, 56] Hence, one could assume that there would be less acid produced after toothbrushing.

Question No. 8: Will toothbrushing prevent the formation of dental plaques?

There is no evidence that toothbrushing as ordinarily performed will prevent the formation of dental plaques.

Question No. 9: Will it remove them from accessible surfaces of the teeth?

Most bacterial plaques can be removed from accessible surfaces of teeth by toothbrushing. However, scientific evidence that toothbrushing can remove all plaques from the accessible surfaces of the teeth is lacking. Bacterial plaques usually re-form rapidly.

Question No. 10: Will it reduce the count of acidogenic bacteria?

There is no evidence that the count of acidogenic bacteria will be reduced for an appreciable time by toothbrushing, although a temporary reduction will result.

Question No. 11: What, if any, is the reduction in the caries attack rate that may be expected from a toothbrushing program?

There is little scientific evidence that a reduction in the caries attack rate may be expected from a toothbrushing program. However, the possibility that adequate toothbrushing may reduce the incidence of dental caries should not be ignored.

CHEWING GUM

Question No. 1: Does the use of commercial chewing gum reduce the number of acidogenic organisms in the saliva?

The committee could find no satisfactory evidence that the use of a commercial chewing gum reduced the number of acidogenic bacteria in the saliva, nor could it find any scientific data which indicated that the chewing of gum might stimulate such organisms. There is conjecture in the literature that chewing gum is both harmful and beneficial, but there is no acceptable evidence to support the conjectures.

Question No. 2: How much sugar may be found in chewing gum?

The sugar content of commercial chewing gum is approximately 60 per cent by weight.

Question No. 3: Is chewing gum a caries-producing agent?

There is little evidence that can be cited to answer the question of promotion of dental caries by chewing gum. According to the only available findings,[58] chewing gum was apparently harmful to one group of the population and harmless to another. Gum which contained calcium carbonate seemed to have a less harmful effect than gum without it. However, the evidence that has been offered is not sufficient to justify any conclusion.

Question No. 4: Is an anticaries agent more effective as a mouthwash than when incorporated in chewing gum?

No comparative studies have been made and much would depend on the agent used for caries control. An agent being slowly liberated from chewing gum would have a more prolonged effect than if used in a mouthwash. However, the stimulation of salivary flow produced by the chewing process might have a serious diluting effect on the inhibiting agent. In the opinion of the committee, much would depend on the nature of the anticaries agent.

Question No. 5: In a lozenge?

There is no satisfactory evidence to show any anticaries superiority or even a retarding influence when the agent is employed in a lozenge. The lozenge would permit a more prolonged contact with the teeth. On the other hand, the danger of toxicity from an agent incorporated either in a lozenge or in chewing gum probably would be greater than in a mouthwash, because it would be swallowed.

Question No. 6: Is there sufficient evidence that fluoride incorporated in chewing gum reduces the caries attack rate?

There is no evidence that sodium fluoride in a chewing gum is effective as an anticaries agent.

Question No. 7: Menadione (synthetic vitamin K)?

There is some indication that menadione may reduce the incidence of dental caries but the evidence currently available is not sufficient to justify any conclusions. In the only available report chewing gum was used immediately after the ingestion of food.[57] Those who chew gum do not always use it in this manner. An objection to the widespread use of this means of treatment might be that many persons are averse to the use of chewing gum for esthetic reasons. Still another possible objection is the tendency of gum to stick to acrylic restorations.

Question No. 8: At what times during the day should either be chewed?

Further study is necessary before any general use of chewing gum is justified.

Question No. 9: Is the human organism likely to suffer a shortage of vitamin K?

Deleted.

Question No. 10: Will chewing gum clean the teeth more effectively than will the foods eaten by civilized persons?

A thorough search of the literature reveals no evidence which would justify the conclusion that chewing gum has a greater cleansing effect on the teeth than the natural foods in the diet of civilized persons.

Question No. 11: Would the use of menadione in chewing gum be harmful?

The available evidence indicates that menadione, used frequently in small amounts, would not be toxic. However, until more extensive studies have been conducted, especially on human beings, no conclusions are justified.

DENTIFRICES

Definition: A dentifrice is a substance designed to help the toothbrush clean the accessible surfaces of the teeth. Some dentifrices may contribute to the establishment or maintenance of a normal structure or function of the mouth.

Question No. 1: Combined with toothbrushing, will any of the dentifrices prevent the formation of plaques on those surfaces of teeth accessible to the bristles?

The reported evidence substantiates the conclusion that dentifrices will contribute to the prevention of plaque formation on those surfaces of the teeth which are accessible to the bristles of the toothbrush. However, the proper use of a dentifrice with a toothbrush will not prevent entirely the formation of some plaques. The literature leads us to conclude that a dentifrice which contains an abrasive is more effective in a given length of time and that the brushing technic is more important than the dentifrice.

Question No. 2: Combined with toothbrushing, will any of the dentifrices remove such plaques?

Removal of accessible plaques has been demonstrated satisfactorily.

Question No. 3: Incorporated in a dentifrice, will dibasic ammonium phosphate reduce the number of oral lactobacilli?

A dibasic ammonium phosphate dentifrice, when used in combination with a like mouthwash, produced a significant reduction in the count of oral lactobacilli. However, it would be desirable to have confirmatory evidence as well as a clinical demonstration of reduction of the incidence of caries.

Question No. 4: Penicillin?

No evidence on human beings is reported in the published literature on penicillin. According to one published article[58] and other reports which have not been published, penicillin, when incorporated in a dentifrice, contributes to the reduction of oral lactobacilli in animals and man. The evidence as yet is insufficient to show that it produces a decrease in the incidence of dental caries in human beings. The use of penicillin dentifrices is an interesting experiment, but until further evidence is available concerning its allergic manifestations and its influence on other bacteria and until it has been demonstrated to reduce the incidence of caries in human beings, its use should be considered purely in the experimental stage.

Question No. 5: Other antibiotics?

There is inadequate evidence on which to base an opinion of the value of other antibiotics in the control of caries, although it is known that oral lactobacilli are in some degree sensitive to streptomycin and to tyrothricin.

Question No. 6: Fluorides?

Only two articles on the use of fluorine in dentifrices appear in the literature.[59, 60] One article reports no reduction in oral lactobacilli and no significant reduction in the incidence of dental caries. The other article reports a reduction in oral lactobacilli, but the evidence that a substantial reduction occurred is not convincing. The committee concludes that the available evidence does not justify the use of fluorine dentifrices.

Question No. 7: How frequently should the dentifrice be used?

There is nothing in the literature that establishes how frequently a dentifrice should be used. However, there is some evidence that plaques which remain in the mouth may become impregnated with inorganic material and thus become difficult to remove. Therefore, one might reason that the more frequently a dentifrice is used, the greater its cleansing effect. In all probability, great variation is found in different mouths.

Question No. 8: How permanent is the effect?

All available evidence points to the conclusion that the effect of currently available commercial dentifrices is transitory.

Question No. 9: Has a reduction in caries attack rate been demonstrated?

If lactobacillus counts can be accepted as an expression of the caries attack rate, the evidence examined demonstrates that there is substantial reduction in the caries attack rate when certain medicated dentifrices are employed. Laboratory evidence has shown a reduction in the incidence of caries when commercial dentifrices were used to brush the teeth of hamsters.[61]

MOUTHWASHES

Definition: A mouthwash is any fluid suitable for use in rinsing the oral cavity.

Question No. 1: Will rinsing the mouth with tap water reduce the oral bacteria per cubic centimeter of saliva?

Rinsing the mouth with tap water may reduce the bacterial content temporarily by mechanical means and may dilute the bacterial substrate. It has not been shown that the effect of tap water is clinically significant.

Question No. 2: What substances, which may be incorporated in mouth rinses, will reduce the number of oral lactobacilli measurably?

Urea (4 per cent) and quinine (0.05 per cent), urea (3 per cent)-diammonium phosphate (5 per cent) rinse, and urea in 40 to 50 per cent concentration.[62]

Question No. 3: Are these substances now toxic to patients?

Relatively, yes. The toxicity of urea is low, the clinical dose permitted being 15 to 60 gm. a day. There is small likelihood that this substance, used in a dentifrice or mouthwash, will cause toxic manifestations. In a series of patients, concentrations of urea up to 95 per cent were used in a dentifrice for several months with no evidence of toxicity or irritation of the oral mucous membranes. If the urea is combined with urease in a dentifrice, as has been reported, irritation of the oral mucosa could result from the rapid conversion of urea into ammonium carbonate.

Question No. 4: Are there any substances which, when incorporated in a mouth rinse, penetrate the dental plaque and inhibit or destroy the activity of acidogenic organisms within the plaque?

Most antiseptics may penetrate the plaque slowly and may be expected to destroy some of the susceptible organisms in the plaque, but this phenomenon has not been shown to result in a reduction of the incidence of dental caries. The available information indicates that some plaque material is not penetrated easily by chemical agents suitable for use in the mouth.

Question No. 5: Do any of these substances disintegrate the plaque?

It has not been shown that any substance which is suitable for use in a mouth rinse will disintegrate the plaque.

Question No. 6: Do dibasic ammonium phosphate, sodium fluoride, urea, zephiran and quinine destroy or reduce the number of acidogenic organisms?

When used as mouthwash ingredients, it is probable that all of the agents mentioned except sodium fluoride may have a measurable effect on the number of oral lactobacilli. It has not been shown conclusively that such effect will result in a measurable reduction in the incidence of caries.

6, a: The question was changed to read: What is the optimum frequency of use?

The optimum frequency is not known.

6, b: How long does the effect maintain after the mouthwash is discontinued?

The available evidence indicates that the effect is transient.

6, c: Is each practical for the patient to use?

Aside from lack of palatability to some patients, they are convenient to use.

6, d: Can a reduction in caries attack rate be demonstrated by the use of any of these agents?

Deleted.

SUMMARY

Maintenance of oral hygiene by means of dental prophylaxis and toothbrushing with acceptable aids can be recommended for numerous reasons, although the evidence that such measures are effective in the prevention of dental caries is limited and, at present, inadequate.

Respectfully submitted: Floyd D. Ostrander, D.D.S., chairman; Donald A. Wallace, Ph.D., secretary and consultant; Philip D. Adams, Ph.D., Lloyd C. Blackman, D.D.S., Glenn E. Cartwright, D.D.S., Robert A. Downs, D.D.S., Henry C. Gage, D.D.S., Maynard K. Hine, D.D.S., Evan C. Howell, D.D.S., Harvey C. Janke, D.D.S., Levi H. Johnson, D.D.S., Gordon A. Smith, D.D.S., Mary L. Soule, M.A., Harry Strusser, D.D.S., Joel Vugteveen, D.D.S., Walter S. Weisz, D.D.S., and Virginia Winterhoff, D.D.S.; Marguerite F. Hall, Ph.D., Thomas J. Hill, D.D.S., and Robert G. Kesel, D.D.S., consultants.

Note: Names were deleted from the original statement and a reference to "aciduric organisms" was changed to "oral lactobacilli." Dr. Teuscher moved the adoption of the report. The motion was seconded by Dr. Janke and received unanimous approval.

Fluorides and Silver Salts in Caries Control

Committee IV considered the use of fluorides and silver salts in the control of caries.

FLUORINE IN WATER SUPPLY

The inverse relation between the consumption of fluoride-bearing domestic water and the prevalence of dental caries in children has been established. The evidence indicates that the D.M.F. rate is approximately 60 per cent lower in children aged 12 to 14 years who, throughout life, have ingested communal water containing about 1.0 part per million of naturally-borne fluorides (F.). Caries reduction is most marked in the upper anterior teeth, in which the number of D.M.F. surfaces may be lowered by as much as 90 per cent. The reduction is markedly less in the posterior teeth. Some caries-inhibiting effect is observed in areas where the communal water contains as little as 0.5 p.p.m. of fluoride. It becomes more pronounced as the fluoride concentration is increased, though little additional benefit is obtained from concentrations above 1.0 to 1.5 p.p.m. Above this level dental fluorosis may become evident.

The evidence indicates that no toxic effect follows consumption of water containing up to 1.0 p.p.m. of fluorides and that no known toxic effect other than a mild dental fluorosis follows ingestion of water in the 1.0 to 1.5 p.p.m. range. Although progressively severe dental fluorosis may be expected at higher levels, systemic fluoride toxicosis has not been demonstrated on the North American continent after continued usage of waters containing up to 4.0 or 5.0 p.p.m. of fluorides.

The mechanism whereby the inhibiting effect is exerted is not clearly understood.

Lactobacillus counts on groups of persons in fluoride areas are consistently lower than the counts of comparable groups in areas where the communal water contains little or no fluorine. Among persons with continuous residence in a

fluoride area teeth with fluorosis are no more resistant to dental caries than are teeth with enamel having a normal appearance. Chemical analyses indicate that mottled enamel has a higher fluoride content than normal enamel and that the enamel of noncarious teeth contains more fluoride than that of carious teeth, even when the teeth under study have been extracted from the same mouth. Evidence is not available to indicate whether the teeth of the child are affected when fluorides are ingested by the mother. There is some evidence that residence in a fluoride area confers some immunity to caries, even in persons who had used fluorine-free water during the period of enamel calcification. Whether artificial fluorination of water will produce the same reduction in caries as naturally occurring fluoride waters is not known.

A statement made in 1942 remains true today: "There is no direct evidence demonstrating whether or not the addition of fluorine to a fluorine-free community water supply will decrease the incidence of dental caries in the community."[63] Studies designed to test the efficacy, safety and practicability of fluorinating a community water supply are now under way in several communities.

TOPICAL APPLICATION

Caries reductions of approximately 40 per cent have been observed in the teeth of groups of children treated topically with solutions of sodium fluoride.

An acceptable technic should include cleaning and drying the teeth and applying a 2 per cent solution of sodium fluoride (C.P.) which is allowed to dry for three minutes. The maximum protective effect is attained after a series of four treatments, only the first of which is preceded by a prophylaxis.

The protective effect of the treatment continues for three or more years. The topical treatment does not produce fluorosis and does not injure the gingival tissues, and fluorine is not absorbed in harmful amounts as a result of the treatment.

As in the case of natural fluoride waters, the mechanism whereby topically applied fluorine exercises its protective effect is obscure.

There is no evidence as to whether or not topically applied sodium fluoride will confer any additional caries protection to teeth which have calcified during residence in a fluoride area.

FLUORINE IN FOOD

Experimental evidence indicates that fluorides in food are less fully utilized than fluorides in water. The evidence does not indicate that foods grown in different geographic areas show any wide variation in fluoride content.

Although bone meal contains fluorine, it has not been established as a good source of this element, and there is insufficient evidence that caries can be controlled either in children or in adults by its use. Nor does the evidence today support the recommendation for the control of caries in children or adults by the use of any other proprietary product containing fluorine for self administration or for home use.

TOXICITY OF FLUORIDES

Fluorine is toxic, but in the strengths and amounts recommended in this evaluation its use in skilled hands is without danger to the patient.

FLUORINE IN PROPHYLAXIS PASTE

On the basis of the evidence of preliminary investigations, there may be some caries-inhibitory effect after the use of sodium fluoride mixed with pumice as a prophylaxis powder or paste.

SILVER NITRATE

It has not been demonstrated adequately that the incidence of dental caries can be reduced or that progress of the caries lesion can be retarded by the topical application of silver nitrate. There is evidence that silver nitrate alters both the organic and inorganic constituents of dental enamel, but there is insufficient evidence that these alterations have any value in the control of dental caries. The use of a wetting agent has no proved value.

Respectfully submitted: Albert L. Russell, D.D.S., chairman; Evans G. Nelson, D.D.S., secretary; A. Paul Atkins, D.D.S., Harry K. Brown, D.D.S., John E. Chrietzberg, D.D.S., Sydney B. Finn, D.D.S., Kenneth R. Gibson, Sr., D.D.S., Edmund A. Grant, D.D.S., John W. Holt, D.D.S., Foster W. Matteson, D.D.S., James F. Owens, D.D.S., Alfred E. Russell, D.D.S., Annie J. Taylor, Edward Taylor, D.D.S., James A. Thrash, M.D., and Chester V. Tossy, D.D.S.; Francis A. Arnold, Jr., D.D.S., Basil G. Bibby, D.D.S., John W. Knutson, D.D.S., and Helmut A. Zander, D.D.S., consultants.

Note: Two minor deletions were made from the original report during the discussion period. It was moved by Dr. Teuscher that the report be adopted. The motion was seconded by Dr. Seyler and received unanimous approval.

Vitamin and Mineral Ingestion

Committee V, in its deliberations and conclusions, has proceeded on the basis of the following premise: Any effects derived from the ingestion of vitamins and minerals must be considered intrinsic in character and manifested, in their relation to dental caries, as (1) variations in the structure, form or composition of the teeth, produced during the period of tooth formation, or (2) variations in the saliva or other factors comprising the environment of the teeth, produced during any period of life.

The committee, after consideration of the evidence submitted by the essayists during the conference and after a limited search of the literature, submits the following general conclusion:

The ingestion of vitamins and minerals, in amounts in excess of those required for a normal diet, has not been demonstrated to have any relation to dental caries.

The committee, concluding that answers to the evaluative questions proposed by the general planning committee would be helpful in serving the several purposes of the conference submits the following additional conclusions with reference to, and in the numerical order of, the specific questions:

I. The vitamins

A. Vitamin A

1. With positive signs of vitamin A deficiency, do children exhibit an increased dental caries attack rate?

There is no evidence that vitamin A deficiency causes an increase in the dental caries attack rate in children.[64-67]

2. During the period of tooth formation, does a mild chronic deficiency of vitamin A affect the structure of the hard tissues or the shape of the teeth? Chronic mild deficiencies of vitamin A have no significant effect on the structure of the hard tissues or the shape of the teeth. However, chronic severe deficiencies of vitamin A produce characteristic abnormalities in the structure and form of the enamel and dentin of developing teeth of animals. It is doubtful whether avitaminosis A of a degree and duration required to produce similar dental disturbances occurs in the infant or could be tolerated by the infant. The only authenticated report of a dental disturbance in a human being that was caused by vitamin A deficiency was reported by Boyle,[68] who examined a premature infant whose nutrition was unusually poor.

B. Vitamin B complex

1. Do children with positive signs of multiple B deficiency exhibit an increased dental caries attack rate?
2. A reduced caries attack rate?

There is no evidence or even a suggestion in the literature that a deficiency of vitamin B or any of its components has any effect on the dental caries attack rate. The vitamin B complex is concerned primarily with the soft tissues, including gingiva, tongue and mucous membrane, and there is no evidence of a relation between vitamin B complex and the enamel or dentin of the teeth.[69-71]

C. Vitamin C

1. What effect, if any, does a deficiency of vitamin C have on developing teeth in man?

There is no evidence in the literature that a deficiency of vitamin C has any effect on the developing teeth of man, but in experimental animals with a deficiency in vitamin C changes occur which result in loss of formation of intercellular substance with development of amorphous and irregular dentin.[72-74]

2. Will the daily ingestion of large amounts of vitamin C reduce the caries attack rate?

That the daily ingestion of large amounts of vitamin C reduces the dental caries attack rate has not been substantiated in the literature. Hanke's experiments were reported to show these results, but his work has not been corroborated.[75-77]

D. Vitamin D

1. Will fortification of the diet with vitamin D during tooth formation reduce the caries attack rate?

2. After tooth formation?
   It has been rather clearly demonstrated that vitamin D is of value in the formation of hard dental structures including the teeth themselves. There is evidence to suggest the possibility of increased susceptibility to caries attack in teeth which have been formed during a condition of vitamin D deficiency.
   Evidence that this vitamin aids in the maintenance of the fully formed teeth or in the prevention or retardation of dental caries at any stage of its progress is not supported by observations that have passed beyond the controversial stage.[78-80]
3. What happens when patients take excessively large doses of vitamin D?
   Recently numerous cases of serious renal disturbances have been reported to have resulted from the administration of massive doses of vitamin D given as therapy for arthritis.[81]
4. Is there any difference in the patient's caries attack rate when he receives his vitamin D as sunshine, as cod-liver oil or as concentrates such as viosterol?
   There is no conclusive evidence to indicate that there is any significant difference in the caries attack rates of patients who receive vitamin D from sunshine, cod-liver oil or irradiated ergosterol.[82, 83]
5. Do people living in civilized urban communities of sunny areas have fewer cavities than those living in comparable communities of cloudy areas?
   Since it is possible that other geographic factors may influence differences in caries attack rates, there is not enough conclusive evidence in the literature to indicate that sunshine is the only factor involved in determining geographic differences in dental caries attack rates.[84-86]
6. Are more hypoplastic teeth found in the mouths of rachitic children than in the mouths of nonrachitic children?
   It is probable that more hypoplastic teeth occur in the mouths of rachitic children than of nonrachitic children. Recent studies, however, suggest that there is no correlation between hypoplasia of the teeth and rickets or vitamin D deficiencies occurring during tooth formation.[79, 80]
7. Ordinarily, is the prenatal portion of the dentin and enamel in erupted deciduous teeth hypoplastic?
   It may be concluded that the prenatally formed portion of the dentin and enamel in erupted deciduous teeth ordinarily is not hypoplastic.[87]

II. The minerals (calcium and phosphorus)

1. Does their ingestion improve tooth formation?
   There is sufficient evidence in the literature to justify the view that the ingestion of calcium and phosphorus in amounts sufficient to

prevent skeletal defects will be adequate for the formation of teeth with normal composition and structure in the average well child. It is possible that certain persons, because of illness or metabolic abnormalities, will require an intake of calcium and phosphorus greater than that needed by the usual child in order to prevent abnormalities of structure, growth and composition of the skeleton and teeth. It appears, however, that forming bone always is more sensitive to deprivation of calcium and phosphorus than are developing teeth.[88, 89]

2. Does their ingestion after the eruption of teeth reduce the caries attack rate?

   There is no conclusive evidence in the literature that the caries attack rate for fully formed erupted teeth is affected by the addition of calcium and phosphorus even to a low-normal diet.[90, 91]

3. Are structurally normal teeth less susceptible to caries than hypoplastic teeth?

   The evidence regarding the relative susceptibility to caries of hypoplastic and structurally normal teeth has not passed beyond the controversial stage. Analyses of the enamel of sound teeth and the sound enamel of carious teeth showed no differences of composition with regard to calcium, phosphorus, magnesium and carbonates.[79, 80, 92]

4. Is there any necessity for a normal child to ingest mineral calcium in addition to his daily quart of milk?

   There is no evidence to indicate that normal children require more calcium than is supplied by a diet adequate to meet total nutritional requirements; such a diet will contain approximately a quart of milk a day.[91]

5. Is there any reason for a normal pregnant woman to do so?

   There is no evidence to indicate that well nourished pregnant women require more calcium than is supplied by a diet adequate to meet total nutritional requirements.

6. Is the organic calcium of milk absorbed more easily than the inorganic calcium salts from tablets?

   There is ample evidence to support the conclusion that calcium of milk is better absorbed than calcium present in any other form.

7. Does the calcium-phosphorus ratio in saliva or blood affect the caries attack rate?

   There is ample evidence to support the view that the content of calcium or phosphorus in blood or saliva or the calcium-phosphorus ratio in either of these fluids has no correlation with the caries attack rate in the human being.[93]

8. Do the teeth of children born to mothers with osteomalacia exhibit a high degree of hypoplasia and caries?

Day,[80] in a study of the incidence of dental caries in children born of women with severe osteomalacia in Northern India, reported that only two of 144 teeth in 7 children with severe rickets were carious.

9. Is rickets a frequent cause of hypoplasia?

It appears that hypoplasia of human teeth frequently is due to the ingestion of a suboptimal amount of vitamin D during tooth formation. It also appears that vitamin D deficiency is not the only cause of enamel hypoplasia. Day[79, 80] found that vitamin D deficiency during infancy and childhood does not always result in hypoplasia of the enamel.

10. During what age period is rickets commonly detected?

Rickets usually occurs before the second year; that is, during the period of most active bone growth and development. Bony residues of the disease may continue to be present throughout the lifetime of the person. Furthermore, there is evidence, recently produced, which shows that a subclinical form of rickets may occur frequently up to 14 years of age.[94]

11. Is calcium from chocolate milk and from whole milk equally assimilable by human beings?

There is no evidence of any difference in the human body's utilization of the calcium from chocolate milk as compared with its utilization of calcium from whole milk.

Animal studies indicate that the rat assimilates a higher percentage of calcium from whole milk than from chocolate milk because of the oxalic acid (0.5 to 0.6 per cent) in cocoa, but the oxalic acid content is not sufficient to disturb the assimilation of calcium by human bodies.[95]

Respectfully submitted: Philip E. Blackerby, Jr., D.D.S., chairman; Hugh M. Tarpley, D.D.S., secretary; Stanley C. Brown, D.D.S., Albert E. Bucholz, D.D.S., Gerard J. Casey, D.D.S., Sarah E. Hill, B. A., Irvin W. Leichtfuss, D.D.S., and William H. Rumbel, D.D.S.; Wallace D. Armstrong, Ph.D., and Adelia M. Beeuwkes, M.S., consultants.

Note: After a period of discussion in which no changes in the statement were made, adoption of the report was moved by Dr. Teuscher, seconded by Dr. Owen and approved unanimously.

Various Types of Diets

Committee VI was assigned the subject "The Contribution of the Type of Diet to Caries Control," with three subheadings: (1) adequacy of the diet, (2) consistency of the diet and (3) carbohydrate restriction.

Members of the committee sought information on the following two points: 1. Has the diet of the people in the United States improved since 1900? 2. Has the dental caries attack rate increased since 1900?

DIETARY SINCE 1900

The per capita food supplies in this country are bountiful compared with those of most other parts of the world. The per capita volume has remained fairly constant during the last three decades, but within the total, the relative importance of various foods has been shifted. There has been a phenomenal rise in the consumption of sugar and citrus fruits, and an upward trend in the consumption of dairy products. From the standpoint of nutrition, certain of these trends in food consumption have enriched the diets of the American people while others have impoverished them. On the credit side, for example, is the increase in consumption of dairy products, fruits and succulent vegetables; on the debit side, the increase in consumption of refined sugar.[96]

DENTAL CARIES ATTACK RATE

Reliable evidence regarding an increase in the dental caries attack rate is meager. If the two studies available to the committee are indicative of national trends,[97, 98] it may be assumed that the dental caries attack rate has increased.

ADEQUACY OF DIET

Although the committee found no conclusive evidence that an adequate, optimal or balanced diet as outlined by the Food and Nutritional Board, National Research Council, would influence the caries attack rate, it is imperative that a diet conducive to individual health be advocated.

The committee uncovered no substantial evidence that fibrous foods, uncooked fruits or vegetables or so-called detergent foods reduce the caries attack rate.

CARBOHYDRATE RESTRICTION

Studies by a number of investigators indicate that the restriction of sugar, either refined or natural, is effective in the control of dental caries.[99-110] Moreover, the restriction of sugar, either refined or natural, will improve the dietary, provided the caloric intake remains adequate.[111] The harmful effects of sugar, refined or natural, are not reduced by the addition of either vitamins or minerals.

Respectfully submitted: J. M. Wisan, D.D.S., chairman; W. F. Hoffman, D.D.S., secretary; Gladys Eyrich, B. F. Gunter, D.D.S., J. C. Livett, D.D.S., B. M. Medof, D.D.S., Elsie Schildwachter, D.D.S., Feno Shafer, D.D.S., and Walter D. Thomas, D.D.S.; Hermann Becks, M.D., D.D.S., Philip Jay, D.D.S., and Genevieve Stearns, Ph.D., consultants.

Note: The discussion resulted in no change in the original statement. The adoption of the report was moved by Dr. Teuscher, seconded by Dr. Chrietzberg and then approved unanimously.

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